Cancer Treatment-Induced Cardiotoxicity: a Cardiac Stem Cell Disease?

L. Prezioso[1,2], S. Tanzi[3] , F. Galaverna[1], C. Frati[1,2], B. Testa[1,2], M. Savi[2,4], G. Graiani[1,2], C. Lagrasta[2,5], S. Cavalli[1,2], S. Galati[1,2], D. Madeddu[1,2], E. Lodi Rizzini[9], F. Ferraro[9], E. Musso[2,4], D. Stilli[2,4], K. Urbanek[10], E. Piegari[7], A. De Angelis[7], A. Maseri[8], F. Rossi[7], E. Quaini[6], and F. Quaini[1,2,*]

Dept of Medicine[1], Pathology [5], Oncology [3], Hematology [9], and Physiology [4]; CISTAC Center [2], University of Parma, Italy; Brigham & Women's Hospital[10], Harvard Medical School, Boston, USA; Dept of Pharmacology [7], Second University of Naples, Italy; Heart Care Foundation, Firenze, Italy[8]; Observatory of the Italian Society of Cardiac Surgery, Rome, Italy[6]

Abstract: Cardiovascular diseases and cancer represent respectively the first and second cause of death in industrialized countries. These two conditions may become synergistic when cardiovascular complications of anti-cancer therapy are considered. More than 70% of childhood and 50% of adult cancer patients can be cured, however this important success obtained by the biological and medical research is obfuscated by emerging findings of early and late morbidity due to cardiovascular events. Although anthracyclines are effective drugs against cancer a dose-dependent cardiotoxic effects whose mechanism has not been elucidated resulting in failure of therapeutic interventions limit their use. Unexpectedly, tyrosine/kinase inhibitors (TKIs) aimed at molecularly interfering with oncogenic pathways, have been implicated in cardiac side effects. Possible explanations of this phenomenon have been ambiguous, further strengthening the need to deepen our understanding on the mechanism of cardiotoxicity. In addition to a detailed description of anthracyclines and TKIs-related cardiovascular effects, the present review highlights recent observations supporting the hypothesis that the cellular target of anthracyclines and TKIs may include myocardial compartments other than parenchymal cells. The demonstration that the adult mammalian heart possesses a cell turnover regulated by primitive cells suggests that this cell population may be implicated in the onset and development of cardiovascular effects of anti-cancer strategies. The possibility of preventing cardiotoxicity by preservation and/or expansion of the resident stem cell pool responsible for cardiac repair may open new therapeutic options to unravel an unsolved clinical issue.

Key Words: Cardiotoxicity, cancer treatment, anthracycline, tyrosine kinase inhibitors, cardiovascular diseases, stem cells, cardiac progenitors, regenerative medicine.

INTRODUCTION

Anthracyclines are active anti neoplastic drugs able to improve survival in hematologic [1] and solid [2] tumors. Anthracyclines containing regimens have shown their potent anti-tumor efficiency since 1969 [3] becoming the gold standard since around 1998. However, their cardiotoxic adverse effects, including cardiomyopathy and congestive heart failure (CHF) [4], severely limit their use.

Women with breast cancer are now considered for the treatment with targeted drugs that cause moderate cardiotoxicity when used as single agents but aggravate clinical manifestations of cardiac dysfunction when used in combination with anthracyclines [5]. Cardiac toxicity of targeted drugs seems to be manageable and reversible at a short- or mid- term follow-up, but the development of late cardiovascular sequelae is more than a speculative possibility. Differences between pediatric, adult, and elderly patients and the lack of uniform modality in detecting and reporting cardiac events make such estimates even more difficult to interpret [6].

The important advances in pharmacological and multimodality intervention of anti-cancer strategies aimed at improving the efficacy and attenuating toxicity have been partially frustrated by increasing reports of systemic and undesired clinical manifestations. Due to the significant increase in the oncologic population of long term survivors which accounts for 60-70% of the cases, we are facing an important epidemiologic problem related to the late morbidity and mortality affecting the quality of life of otherwise successfully treated cancer patients.

In this review, by providing detailed information on drug-related cardiovascular complications and new insights on the pathogenetic mechanisms of cardiotoxicity of antineoplastic therapy, an attempt was made to open innovative prospectives to impact on an emerging relevant clinical issue.

*Address correspondence to this author at the Department of Medicine, University of Parma, *Via* Gramsci, 14, 43100 Parma Italy;
Tel: +39/0521/033297; Fax: +39/0521/033271;
E-mail: federico.quaini@unipr.it

1871-5257/10 $55.00+.00

The recently introduced, although timely known, concept that the heart is not a post mitotic organ [7, 8] and contains a stem cell pool responsible for myocardial cell turnover [9, 10] may offer a new way in understanding cardiotoxicity. Specifically, beyond cardiomyocytes or parenchymal cells, an alternative cellular target of anticancer therapy that is the progenitor cell population controlling myocardial homeostasis is proposed to explain a major clinical issue in oncology.

Due to their well established or newly discovered cardiotoxic effects, two major classes of antineoplastic drugs will be analysed here, respectively, anthracyclines and tyrosine kinase inhibitors (TKIs).

ANTHRACYCLINE

General Considerations

Anthracycline- induced cardiotoxicity is irreversible and directly related to the cumulative dose [11]. Anthracycline toxicity is usually classified as early or late depending on the time of occurrence after administration [12-14]. Immediately after treatment initiation, reversible arrhythmias, pericarditis and hypertension can occur [15]. High dose impairs systolic function and causes CHF [16]. The chronic cardiomyopathy can appear even after several decades from the treatment with a progressive deterioration of left ventricular function, myocyte death and irreversible cardiac failure in at least 20% of the cases [17]. In some individuals, rapid deterioration in cardiac function takes place even at low dose and more than 50% of patients receiving a total dose of 300 mg/m^2 experienced a reduction in Left Ventricular Ejection Fraction (LVEF) of < 30% and occurrence of CHF [18]. Thus, a sharp distinction between acute and chronic toxicity may be artificial because anthracycline-induced damage can take place with the first dose suggesting that cardiotoxicity could be more accurately defined as a progressive condition in patients receiving the drug.

The incidence of CHF is as high as 26% in patients treated with 550 mg/m^2 of anthracyclines [16] but the alterations in LV function can be detected with much lower doses, highlighting the subclinical (i.e. asymptomatic) cardiac damage that eventually progresses with time.

Cardiac abnormalities that develop during a long follow up period after anthracycline therapy may be exacerbated in middle age and elderly patient who are already prone to cardiac diseases. General risk factors for cardiovascular diseases such as hypertension, hyperlipidemia and diabetes may contribute to the progression of cardiac damage [19]. Smoking similarly causes a severe oxidative stress that increases the risk of cardiovascular diseases, especially in women [20, 21].

Female sex is generally associated with a remarkable, about two-fold, increase in cardiac morbidity and mortality once a cardiac risk factor is established [22]. Interestingly, the increased susceptibility to anthracycline in female is also observed with diabetes [23, 24] and after thrombolytic therapy [25] although primary cardiac events prevail in male. Older women and younger women show similar reductions in mortality due to chemotherapy for breast cancer, and age alone should not be a contraindication to the use of chemotherapy in older women who are in good health [26, 27]. However, few studies have been focused on the impact of sex on cardiotoxicity. Thus, a discussion on this issue together with a proposed pathogenetic mechanism is detailed in the paragraph Pediatric Population.

It has also been clearly documented that local radiation therapy increases the risk to develop cardiovascular pathologies [28], strongly supporting the contention that alterations of the resident myocardial cell turnover is an important factor in the development of cardiotoxicity.

Importantly, recognition of genetic and proteomic markers of individual patient susceptibility to the cardiotoxic effects of anthracyclines could improve the safety of this treatment [6].

Dual Biochemical Mechanism of Toxicity

Anthracycline possesses at least two different mechanisms of action: a free radical mediated pathway implying a reduction of antioxidant enzyme concentrations and increased oxidative stress possibly responsible for the cardiomyopathy; a non free radical mediated action, with more prominent antitumor effects *via* intercalation with DNA, leading to the formation of a topoisomerase II complex that impairs DNA replication. Anthracycline also causes cardiac injury by triggering apoptotic cascades [29].

A detailed review on the fine biochemical mechanism of action of DOX is extensively addressed elsewhere [4]. Briefly, the current thinking is that anthracyclines may become cardiotoxic after one- or two-electron reductive activation. One electron reduction of the quinone moiety of doxorubicin (DOX) results in the formation of a semiquinone free radical, which regenerates its parent quinone by reducing molecular oxygen to superoxide anion (O2•-) and hydrogen peroxide (H_2O_2), members of the broad family of reactive oxygen species (ROS) known to cause oxidative stress and energy depletion in cardiomyocytes. Two electron reduction of the side chain carbonyl moiety converts anthracyclines to secondary alcohol metabolites [DOX(OL), EPI(OL), etc.] that are slightly less active at redox cycling but remarkably more potent at dysregulating calcium and iron homeostasis. Oxidative stress, iron dysregulation and concomitant alterations of the cardiac-specific gene expression program eventually conspire at inducing cardiomyopathy. Secondary alcohol metabolites therefore accumulate in the heart and form a long-lived reservoir, which may help to understand how anthracyclines introduce a lifelong risk of cardiotoxicity. The cellular pharmacokinetics of secondary alcohol metabolites may also help to explain how anthracycline cardiomyopathy is exacerbated by chemotherapeutic agents that cause little or no cardiotoxicity per se. These results uncover the fact that anthracycline cardiotoxicity would be aggravated by any concomitant drug that stimulated the formation of secondary alcohol metabolites. It is also tempting to speculate that the delayed cardiac events observed in adult or childhood cancer survivors may originate from a toxic synergism of de novo events with the long-lived cardiac reservoir of secondary alcohol metabolites [4].

In spite of these relevant lines of evidence, it should be pointed out that the clinical impact of the role of oxidative

stress in cardiotoxicity is increasingly questioned [6]. One reason for this uncertainty is the apparent lack of protection provided by antioxidants. Although the protective effects of carvedilol, alfa 1 and beta 1,2-adrenoceptor blocker, were tentatively attributed to its antioxidant properties, confirmation awaits comparison of carvedilol with other adrenolytic agents without antioxidant properties [30-32]. The only compound consistently found as cardioprotective in experimental and clinical studies is the iron chelator dexrazoxane [33-35]. However, this drug does not directly inactivate free radicals but attenuates their formation through intracellular iron chelation.

A preliminary question that might be asked is whether the mechanism by which DOX has an effective anticancer action is also the mechanism of cardiac toxicity.

The cytostatic effect of DOX is attributed to intercalation of the planar anthracycline ring structure into the double helix of nuclear DNA to interfere with DNA replication and transcription, especially in rapidly dividing cells [4]. It has been claimed that myocardial cells are mitotically inactive, and therefore the DOX cardiotoxicity ought to be related to mechanisms other than its cytostatic effect [15, 36]. However, this paradigm has been shifted by different methodologies [7-9] and by several laboratories [7, 8, 10] providing strong evidence of the presence of a cardiomyocyte turnover in the human heart controlled by a resident stem cell population.

Cardiac cell turnover has been recently evaluated by a mathematical modelling of ^{14}C integration into DNA of cardiomyocytes from individuals born both before and after the nuclear bomb tests [8]. By this approach, the annual rate of cardiomyocyte renewal involves 1% of the cells at the age of 25 to decrease to 0.45% at the age of 75 [8]. Higher values were observed in noncardiomyocytes since an average cell turnover of 18% was calculated. When proliferating cardiomyocytes were analysed at tissue level in the normal human heart by specific nuclear proteins it was found that nearly 600 and 14 myocytes per million of cells were, respectively, cycling and dividing [37, 38]. Apoptotic cell death involves more than 150 cardiomyocytes per million of cells [39], although the estimation of the cumulative cell dropout may be markedly influenced by higher values of necrotic death normally involving cardiomyocytes of the normal and pathologic heart [40, 41].

Thus, in steady state a small fraction of cardiac myocytes is cycling and may be sensitive to the interference of anthracyclines on DNA replicative machinery. It has also been shown that this population of proliferating myocytes markedly increases in the damaged human heart subjected to overload or ischemic insult [37, 38]. The same phenomenon may be operative in anthracycline induced cardiomyopathy so that repeated administrations of the drug will sequentially affect the population of progenitor and parenchymal cells that are activated in the attempt to repair the damaged myocardium. This would result in a time dependent accumulation of cardiac injury, which is the consequence of both increased cell death and impaired cell replacement. This sequence of cellular events may explain why a cumulative dose of DOX has been recommended to avoid precipitation of cardiac function. In addition, the high incidence of late cardiotoxicity in the pediatric population of cancer patients may well be explained by the negative consequence of DOX on a higher fraction of proliferating cardiomyocytes characterizing the young heart [8, 42]. Thus, the more active turnover, which physiologically dictates cardiac growth at this age, is translated in an increased susceptibility to cytotoxic insult and progressive cells death until a critical number of contractile cells unable to respond to subclinical demand is reached and overt heart failure develops.

Similar cellular mechanisms of tissue damage have been clearly documented in organs or systems provided by high cell turnover such as the bone marrow (BM), where repeated chemotherapeutic regimens can result in irreversible myelotoxicity if the time to recovery for cell replacement is not respected.

Another question, which bears on the mechanisms of toxicity, is why the therapeutic action of DOX should be preferentially associated with myocardial damage with respect to other organs. One possible answer is that the heart is a high-energy consuming organ and therefore needs a continuous supply of ATP. A major factor in cardiotoxicity appears to be an impaired metabolism of high-energy phosphate production due to an abnormal mitochondrial function [43]. Metabolic abnormalities include modifications induced by DOX on ATP and phosphocreatine, in the mitochondrial production of ATP, in the utilization of cardiac substrates, in the storage and transport of CK energy and in the monitoring of signalling of the AMPK sequence [43]. Indeed, available evidence points to the involvement of mitochondria as the principal targets in the development of DOX cardiac toxicity. Nuclei and mitochondria have been shown to be exclusive sites of DOX localization [44]. The early stages of DOX cardiomyopathy involve morphological and functional changes in heart mitochondria, including interference with mitochondrial calcium homeostasis at subclinical cumulative doses [43]. Thus, growing evidence correlates the onset and severity of DOX cardiotoxicity with disturbance in heart mitochondrial function and bioenergetics.

The fact that both DOX and TKIs [5] possess the elective property to alter mitochondrial structure and function may be a converging basis to understand the common associated cardiovascular events. Moreover, it should be mentioned that interaction between the HER-2 signalling pathway and regulation of sarcomere stability is an emerging alternative to the oxidative stress hypothesis of anthracycline cardiotoxicity [45].

Cellular Targets

It is undisputed the fact that cycling or non-cycling cardiomyocytes are directly affected by DOX. The earliest ultrastructural changes occur in sarcoplasmic reticulum (SR) and mitochondria with myofibrillar degeneration occurring later [46]. There is a vacuolar degeneration and swelling of the SR, disruption of mitochondria, and eventually disorganization of myofibrils. Similar lesions are found in the biopsies of patients with DOX-induced cardiomyopathy [47]. Later on, large vacuoles displace both contractile elements and mitochondria. Mitochondria exhibit positive calcium

staining and electron dense bodies suggesting calcium inclusions [46].

However, as mentioned above, the antineoplastic action of DOX has been attributed to the prevention of DNA replication in rapidly dividing cells, a mechanism that would not participate in toxicity of the large fraction of quiescent cardiac myocytes present in the adult heart.

We propose working hypotheses on the cellular mechanism of cardiotoxicity by anti-cancer drugs. Stem cells in the heart are believed to provide replacement for dying myocytes [9] and cardiac toxicity of DOX might include also an inhibition of stem cell proliferation, which is activated by the loss of parenchymal cells, resulting in an impairment of myocytes replacement. The potency of DOX on cancer reproducing cells suggests a preferential toxicity on the multiplying cardiac progenitor cells than adult myocytes by acting on DNA replication. Moreover, impaired high-energy phosphate metabolism might also affect negatively the function of all cardiac compartments including stem/progenitor cells.

The implication of this mechanism would be that in young patients the damage to stem cells might impair their growth therefore contributing later on to cardiac failure. The depletion of high-energy phosphates could be the result of a direct action of DOX on mitochondria and of indirect effects due to the dysfunction of ATP requiring structures of stem cells. This series of events is consistent with the fact that prior to cardiac failure, there is evidence of a compromised systolic and diastolic function in patients treated with DOX [18]. A diminished uptake of calcium into the SR on the one hand would affect force development in systole and on the other it would increase diastolic calcium and impair diastolic relaxation. As cytosolic calcium increases above certain levels it may lead to apoptosis, consistent with the fact that in DOX-induced cardiac failure no hypertrophy occurs [48]. These biochemical events acting on stem cells would be expected to contribute markedly to the lack of compensatory reactions. Therefore, the irreversibility of cardiac failure once it appears might be related to the inability of stem cells to counteract to some extent myocardial damage through cell multiplication and to a chronic ATP deficiency and calcium overload that severely impair stem cell function.

The hypothesis that toxicity of anti-cancer therapy involves cardiac progenitor cells is important also under the contention that stem cells hold the promise of regenerative therapies such as those used for myocardial infarction [49]. Thus, it is conceivable that they might eventually be used in the DOX cardiomyopathy to restore viable myocytes. In this regard, results on rats, recently published and presented at the American Heart Association Congress in 2008, have shown that precocious dysfunction of cardiac stem cells occurs after drug exposure and that the DOX-induced cardiomyopathic heart can be rescued by cardiac stem cell injection [50].

Thus, the possibility of preventing cardiotoxicity should be tested by preservation and/or expansion of the resident stem cell pool responsible for the repair of the pathologic heart. However, long-term animal and clinical studies are needed to advance our understanding of how these cellular mechanisms might influence the lifetime incidence of anthracycline cardiotoxicity.

Pediatric Population

Since childhood cancer survivors are an increasing population with longer life expectation, pediatric patients deserve a separate description.

The introduction of anthracycline as antineoplastic therapy for childhood cancer is an effective success of the modern medicine. During the past three decades, childhood cancer mortality declined dramatically, and it is estimated that 5 years survival rate involves currently over 70% of the children diagnosed with cancer [51]. Unfortunately, this important success is partially obscured by the long-term morbidity of these patients. The CCCS (Childhood Cancer Survivors Study) showed that in a cohort of cancer survivors, 30 years after treatment, the incidence of severe, disabling, or life-threatening conditions or death due to a chronic disease, reaches a cumulative value of 42% [52]. It has also been shown that the cumulative incidence of clinical heart failure, in a cohort of 607 children treated with anthracyclines between 1976 and 1996 with a mean follow-up of 6.3 years, was 2.8%, and the estimated risk increases from 2% after 2 years to 5% after 15 years [53]. Thus, the overall incidence of DOX related cardiac abnormalities seems to increase with time and may involve a significant fraction of adult survivors.

A systematic review of the current literature and the analysis of the results of 25 studies of asymptomatic survivors of pediatric cancer previously treated with anthracycline has been published [54, 55]. A wide variation from 0% to 57% on the incidence of subclinical cardiotoxicity was observed, with 13 studies showing values of more than 20% [55]. These data may have even underestimated the phenomenon since anthracycline-induced cardiotoxicity was not the major objective of the reported studies and the guidelines for the long-term follow-up of such patients were still lacking. Although cardiac abnormalities have been carefully described, there has been only little investigation on the true incidence of asymptomatic disease and there is virtually no evidence regarding the value of any treatment in altering the natural history of cardiac disease in asymptomatic survivors.

Cardiotoxicity manifests itself into three different clinical pictures: acute, early onset chronic progressive and late onset chronic progressive [54, 56-58]. As in the adult, anthracycline-induced cardiotoxicity in childhood can be variable and includes asymptomatic electrocardiographic abnormalities, mild hypotension, arrhythmias, myocarditis, pericarditis, acute myocardial infarction, heart failure, and long-term cardiomyopathy.

Acute anthracycline cardiotoxicity is estimated to be less than 1% [57] and arrhythmias (especially sinus tachycardia) are the predominant clinical sign. Other possible symptoms can be a transient depression of myocardial contractility, or other electrocardiographic changes. Symptoms usually resolve with the discontinuation of therapy.

Early onset chronic cardiotoxicity speaks for a left ventricular dysfunction, which occurs within one year after anthracycline therapy [54, 57]. It may be progressive leading

thracycline therapy [54, 57]. It may be progressive leading to a clinical heart failure. The incidence of early onset chronic cardiotoxicity during the first year of treatment was found to occur in 1.6% of all the patients treated from 1974 to 1990 [57].

Late onset chronic progressive cardiotoxicity occurs more than 1 year after anthracycline therapy [54, 57]. In a cohort of 115 survivors of childhood acute lymphoblastic leukemia it was found that almost 65% presented increased left ventricular afterload or decreased contractility 6 years after therapy [33]. In another study, examining 151 pediatric long-term survivors (up to 25 years) treated with anthracycline, 43% had abnormalities of systolic function and a 9% incidence of cardiac abnormalities requiring treatment was observed [59].

Several studies aimed at the characterization of cardiac remodelling demonstrated that long-term survivors of childhood cancer have impaired myocardial growth and a progressive increase in left ventricular afterload accompanied by LV mechanical dysfunction. The DOX induced cardiomyopathic heart seems to involve both depressed contractility and increased afterload, which results from a decrease of both ventricular mass and wall thickness [60].The loss of myocytes associated with anthracycline therapy leads to LV wall thinning and brings to a progressive LV dilation as shown by echocardiography [61]. However, left ventricular dimension may be increased, normal, or decreased [61].

Compared to the adult population, it is interesting to see how in pediatric patients cardiac abnormalities develop with time after therapy. While adults usually manifest chronic dilated cardiomyopathy with depressed LVEF in response to anthracycline, children develop a dilated cardiomyopathy, which eventually progresses to a restrictive cardiomyopathy [60]. This seems to be the result of a progressive unbalance between the inadequate left ventricular mass responsible for the depressed ventricular contractility and the increased afterload in spite of a normal or even decreased blood pressure. Chronic increase in afterload may play a more important role than the decreased contractility. Indeed, heart failure with preserved LVEF is increasingly recognized in long-term follow-up of adult survivors [33, 59].

The most extensive and detailed study on DOX induced cardiotoxicity in children has been reported by Lipshultz *et al*. [61]. Patients with a median age at diagnosis of 4.8 years, median follow-up after completion of DOX of 11.8 years and median DOX cumulative dose of 352 mg/ m² were examined. Serial echocardiograms were performed to a large number of long-term survivors from acute lymphoblastic leukemia and functional and anatomical parameters were analysed. LV contractility was significantly depressed shortly after DOX therapy, normalized during the next 6 years and then became significantly depressed at 12 or more years after diagnosis. LV end-diastolic dilatation took place during therapy, however it returned to normal values in the low dose DOX-treated group. LV mass was nearly normal soon after therapy, then decreased significantly 6 to 9 years after diagnosis to became permanently decreased at later time intervals. LV wall thickness relative to body/surface area was significantly reduced at the completion of DOX, became thinner over time and was significantly less than normal at all points of follow-up beyond 6 years. Importantly, thinning of the wall was not a dose-related phenomenon. LV fractional shortening was significantly depressed early after therapy and, following a subsequent transient improvement, remained depressed in a dose dependent manner. Similarly, LV afterload, as measured by end systolic wall stress, increased significantly in a dose-dependent fashion. In association with these echocardiographic findings, significant decreased in systolic and diastolic blood pressure over time were documented 9 years after treatment, suggestive of a reduced cardiac output.

Several studies aimed at the identification of probable risk factors for childhood cardiotoxicity have been reviewed [60, 62]. It has been assumed that the combination of multiple factors could lead to a substantial susceptibility to early anthracycline cardiotoxicity. This is a relevant issue since it is clear that early acute anthracycline cardiotoxicity represents per se a risk factor for the future development of cardiac failure. Concomitant radiation treatment is also associated to increased cardiotoxicity, although we do not know whether this effect is synergic or additive [54, 56, 63].

Since the initial studies, it was clear that higher cumulative anthracycline doses were associated with an increased risk for the development of cardiovascular pathologies [33, 56, 58, 60]. As mentioned in the General Consideration chapter, it must be also said that there is no dose of anthracycline that can be surely safe and even lower doses can cause cardiac dysfunction [61].

An important risk factor is represented by younger age at diagnosis since anthracycline therapy before age 4 was significantly predictive of late cardiac dysfunction. [33, 58, 60]. An additional relevant observation about the proneness to develop cardiotoxicity indicates the female sex as an independent risk factor for the development of cardiotoxicity [60]. Echocardiographic evaluation of 120 children and adults who received anthracycline therapy 8 years earlier showed that LV dilatation, reduced LV mass, and decreased systolic blood pressure were more frequent in female patients. Forty-five percent of the female patients had depressed contractility, as compared to 12% of male. According to the results of the study, the relation between sex and the cumulative dose were interactive: the higher the cumulative dose, the greater the difference in contractility between female and male patients. Differences in body composition could be involved to clarify this phenomenon, which still lacks convincing explanations [64, 65].

Understanding the role of sex on the physiologic and pathologic cardiovascular system is complex. Studies on the senescent human and murine heart indicate that the physiologic cell turnover is different in female, lacking the progressive parenchymal cell loss, which is present in male [66-68]. Our hypothesis is that female sex represents a risk factor for cardiotoxicity since in women the favourable anatomical, structural and cellular parameters may result in an impaired cardiac adaptation to a severe insult brought about by cytotoxic drugs. Thus, reparative processes are downregulated in the female heart possibly leading to an inadequate prompt

availability of progenitor cells to respond to high level of oxidative stress.

In this regard, a paradigmatic and dramatic case came recently to our attention during the research project on alternative mechanisms of cardiotoxicity, in which we are collecting cardiac samples from patients who died as a consequence of antineoplastic treatment.

A Caucasian woman at the age of 17 was diagnosed as having NHL lymphoma involving the left lung. She was successfully treated with DOX containing regimens reaching a cumulative dose of 350 mg/kg in association with local radiotherapy (36 Gy). Being free from the disease, at the age of 23 she delivered a healthy boy without post-partum complications. At the age of 43, she started to display symptoms of exertion dyspnoea and moderate fatigue although no medications were prescribed. Following a brief period in good performance status, a progressive impairment of cardiac function took place leading to overt heart failure requiring several pharmacological interventions and hospitalizations. She came to our observation at the age of 47 in bad clinical conditions. A thoracic CAT scan showed a complete collapse of the left lung with consensual dislocation of central mediastinal organs. Moreover, pleural and pericardial effusions together with calcific pericarditis were documented Fig. (**1A** and **B**). Due to failure of intensive pharmacologic treatment, consultation of several cardiac surgeons excluded the indication to any intervention because of the severe clinical picture associated with the inconceivable anatomical condition. After a short period of intensive care the patient died at the age of 48.

The autopsy was performed and, beside a thick calcified pericardium and a complete collapse of the left lung, the heart showed macroscopic parenchymal damage. The histologic examination documented scattered acute and chronic injuries with a marked myocardial fibrosis Fig. (**1C** and **D**). High level of apoptotic and necrotic cell death together with DNA oxidative damage were present in all myocardial cell compartments (data not shown).

Thus, a long term disease free survival obfuscated in this female pediatric patient an ongoing silent cardiac pathology culminating in heart failure 31 years after treatment.

More studies are needed to understand the background of these observations; furthermore, the study of the different hormone regulation in male and female patients, particularly due to Growth Hormone (GH) or sex steroids, or the possible

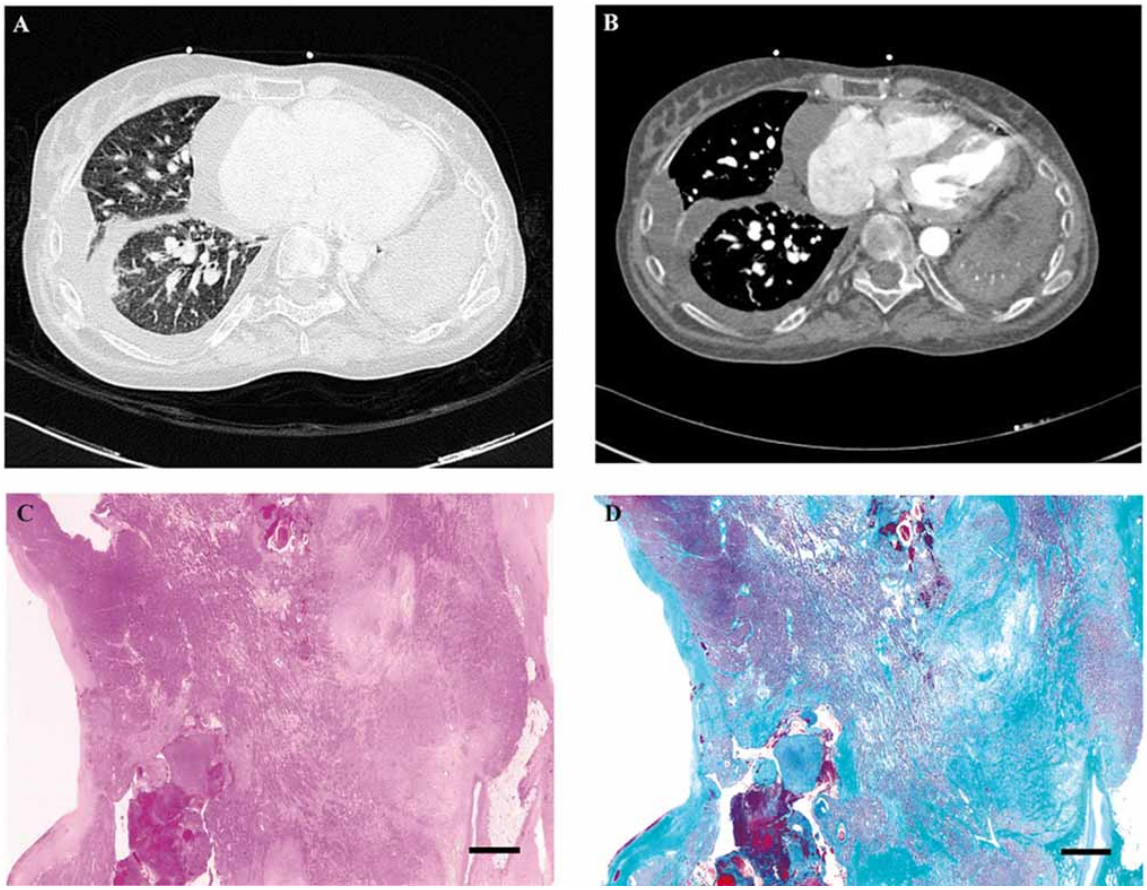

Fig. (1). CAT scan images and myocardial histology of a patient who died 31 years after chemo-radiotherapy (see text for details). **A** and **B**: images taken by CAT scan one month before death corresponding to the parenchymal and mediastinal window, respectively, of the same thoracic section. Complete collapse of the left lung, dislocation of mediastinal organs, pleural and pericardial effusion and calcific pericarditis are illustrated. The right lung is also compromised and shows scissural thickening. **C**: light microscopy of H&E stained section of the heart obtained at autopsy from the same patient, documenting foci of acute damage and extensive myocardial fibrosis best seen in **D** after Trichrome Masson staining of the serial section. Scale bars=200 μm.

differences between the pre-puberty and adolescent treated patients could be an object of great interest for future research.

Detection and Monitoring

Since cardiac repair is limited or, as suggested by our central hypothesis, directly affected by therapeutic strategies, drug-induced cardiac damage is irreversible. Early detection of the cardiomyopathy is therefore of paramount importance.

Subclinical damage as evidenced by cardiac function abnormalities (decreased LVEF) is often evident in anthracycline–treated patients long before clinically overt CHF. Echocardiography is the most common non-invasive method to measure LVEF and is useful to identify patients with significant cardiac dysfunction. However, the definition of cardiac toxicity by an absolute decrease of 10 units in LVEF below its normal value of 50% (used as a strict criterion for discontinuing chemotherapy) [69], may be substantially flawed and may not truly represent the incidence of this phenomenon. It is well established that a decrease in LVEF occurs when the highly efficient compensatory machinery of the myocardium has been impaired; therefore, a decline in LVEF is actually a marker of advanced damage [70]. Additionally, CHF, a condition difficult to diagnose during chemotherapy, frequently occurs even in patients with normal LVEF. As a result of these contentions, a major interest is to explore the clinical utility of alternative more reliable measurements of changes in cardiac integrity.

Methodologies employed to detect clinically apparent cardiac injury include multigated radionuclide (MUGA) scans, identification of various cardiac specific biochemical markers (i.e. BNP and cardiac troponins) and myocardial tissue biopsy.

The American Heart Association (AHA) and the American Society of Nuclear Cardiology, recommends radionuclide angiographic measurement of ventricular function during anthracycline therapy timed 10–14 days after the last dose [71]. It has been stated that is safe to continue therapy if LVEF remains above the lower normal limit or if subnormal LVEF stabilizes after anthracycline therapy is discontinued. A critical review of the data based on these recommendations raised doubt about the reliability and sensitivity of monitoring LVEF for identification of early cardiotoxicity. [6] These recommendations are based mostly on retrospective analysis with a substantial lack of data on the post-anthracycline period. Current monitoring recommendations should therefore be revised [72]. Feasibility studies of new cardiac surveillance modalities would be valuable to clinicians treating patients with anthracyclines or other potentially cardiotoxic chemotherapeutic agents [73, 74]. In this respect, preclinical evidence for a higher diagnostic value of myocardial strain and strain rate as detected by Doppler echocardiography needs to be validated as a surrogate end point before such determinations could be routinely employed for monitoring anthracycline cardiotoxicity [75-77].

The AHA recommends that both children and adults receiving potentially cardiotoxic chemotherapy undergo echocardiography at baseline and re-evaluation recurrently to monitor for changes in cardiac function [56]. Echocardiography, including Doppler analysis, M mode echocardiography, two dimensional transthoracic echocardiography, appear to be useful and non-invasive tests; many studies have confirmed their contribution in monitoring the cardiac dysfunction, although there is still no established regime to measure cardiac risk on survivors of childhood cancer who received anthracycline [54, 56, 62, 78].

Cardiac biomarkers may be best used to identify high-risk patients who are receiving chemotherapy or to more accurately predict cardiac toxicity. Broad application of biomarkers as a less expensive and more accurate tool is still under active investigation [31, 79]. Troponin I (TnI) is released from myocytes in response to cellular damage. Plasma TnI levels increased after anthracycline administration in breast cancer patients after high dose chemotherapy (HDC); this increase was shown to be significantly associated with corresponding decrease in LVEF [80]. Furthermore, follow up echocardiography showed a greater decrease of LVEF in TnI positive compared with TnI negative patients. Moreover, in consideration of the high negative predictive value of TnI (99%), we can certainly identify low-risk patients who do not require close cardiac surveillance after HDC. In contrast, TnI positive patients have a greater incidence of adverse cardiac events.

Elevation of circulating levels of Brain (B)-type natriuretic peptide (BNP), and N-terminal proBNP (NT-proBNP) have shown promise as accurate predictors of cardiac toxicity [70, 81]. Preliminary results suggest that NT-proBNP, a biomarker of unhealthy heart muscle, may also be considered a sign of cardiac stress before irreversible damage occurs in children treated with anthracycline [56]. According to other investigators, NT-proBNP increases early after the first course of anthracycline therapy and higher levels are directly related to the dose of the drug. Though, no relation between increased NT-proBNP and clinical or echocardiographic evidence of cardiac dysfunction was found [82]. Another recent study detected abnormal levels of NT-pro-BNP in 13% of 122 asymptomatic long-term survivors of childhood cancer [83].

Although all of the above results may be promising, a longer follow-up of cancer survivors is needed to confirm or modify the role of biochemical markers in the evaluation of the cardiovascular risk and prediction of heart diseases following anti-cancer therapy.

Treatment

Certain cardioprotective medications have demonstrated benefit in ameliorating the cardiotoxic effects of anthracyclines and HDC [31].

Concerning the rate of administration, prolonged infusion therapy has been recommended to reduce the peak serum concentrations of anthracyclines. This practice became standard, but is not related to an effective advantage as a randomized trial [84] confirmed that a continuous doxorubicin infusion over 48 h did not offer a cardioprotective advantage over bolus infusion, and, on the other hand, could increase hospitalisation and costs, and may create additional stress. Off note, both ways of administration caused cardiac abnormalities.

Early treatment with enalapril, an angiotensin converting enzyme inhibitor (ACE-I), in patients with evidence of myocardial cell injury after HDC seems to prevent the development of cardiotoxicity and the occurrence of associated adverse clinical events [85, 86]. The transient value of ACE-I treatment has been noted in childhood cancer survivors with anthracycline cardiotoxicity, but there is no evidence for their long-term therapeutic benefits [87-90]. In a study involving 18 children who had regular echocardiographic examinations during enalapril therapy, a progressive improvement in LV dimension, fractional shortening and mass, and a decreased afterload was observed. However, all these parameters deteriorated between 6 and 10 years, while LV wall thickness progressively decreased through the whole study [88]. The short-term improvement was transient and primarily related to a decreased diastolic blood pressure, which was identified as the only effective benefit of ACE-I. The lack of preventing the progression of LV dysfunction and wall thinning eventually led to cardiac transplantation or death.

It is unclear whether prophylactic therapy with cardioprotective agents will be helpful in preventing cardiac toxicity in all high-risk patients receiving anthracyclines. A significant interest has been raised by dexrazoxane for cardioprotection during anthracycline chemotherapy [34]. Dexrazoxane is a *bis*-ketopiperazine, which diffuses in cardiomyocytes and hydrolyses leading to the formation of a diacid-diamide with strong iron-chelating properties. As it is known that iron potentiates the toxicity of anthracycline-derived reactive oxygen species (ROS) and that secondary alcohol metabolites further alter iron movements in the cell, an iron chelator like dexrazoxane would be the perfect candidate to mitigate anthracycline cardiotoxicity. Although the cardioprotective effect of dexrazoxane has been documented [91] neither a consensus agreement nor high level of recommendation has been reached. Moreover, one clinical trial has suggested its possible interference with objective tumor response [92] pinpointing to an important issue related to the difficult goal to preserve normal cells from the desired toxic action of antineoplastic therapy on tumor cells. Many other trials uniformly failed to document an interference of dexrazoxane with anthracycline activity nor an interaction between dexrazoxane and anthracyclines was clearly demonstrated in tumor cells [34, 35]. No data, however, are available about the long-term effects of dexrazoxane so that there is still no evidence of improvement in rate of survival for childhood cancer.

However, prompt beneficial responses to cardiac medications may not be associated with sustained cardiac recovery because early cardiotoxicity is a strong predictor of late cardiovascular events in anthracycline-treated long-term survivors of cancer [79]. Thus, once irreversible damage occurs even clinically positive therapies may fail to prevent late cardiovascular complications.

In light of the growing number of cancer survivors, there is a great need of identifying effective interventions after anthracycline treatment that would result in evidence-based recommendations [6]. To mitigate the cardiotoxic risks associated with anthracyclines, studies are in progress to examine the efficacy of nonanthracycline-containing regimens [2, 93].

Liposomal anthracyclines have been developed to reduce DOX cardiotoxicity while preserving its antitumor efficacy. The changes in tissue distribution of liposomal anthracyclines lead to less drug exposure in sensitive organs. Also, the release of the drug is slow, which may avoid high peak plasma concentrations [94]. Pegylated liposomal doxorubicin can improve cardiac safety. In one randomized control trial, 509 patients with metastatic breast cancer received cumulative doses of at least 500–550 mg/m^2. The overall risk of cardiotoxicity was significantly higher with DOX than with the pegylated liposomal form in the presence of similar overall survival [95].

More recently a formulation of epirubicin has been produced in which a nitric oxide (NO) release moiety has been constructed [96]. Results in mice are encouraging and have indicated that by this approach the anti-neoplastic activity of anthracycline is increased in the presence of significant cardioprotection.

Finally, clinical trials are undergoing to determine whether a molecular modification of DOX, consisting in an albumin-binding prodrug, effectively improve anti-tumor efficacy and reduce toxicity [97].

There is no specific actual treatment for DOX-induced cardiac dysfunction. Thus, it appears to be necessary to find new preventive strategies [93].

In this regard, an intuition, which Lipshultz *et al*. converted into a study in 2005, was about the possible benefit of Growth Hormone (GH) therapy for cancer survivors [98]. GH exerts its effect on the heart through Insulin-like Growth factor 1 (IGF-1), to maintain an adequate LV mass. GH deficiency is a common problem for children who survive a childhood cancer. During this study, GH-treated children (cancer survivors with a developed GH-deficiency) were compared to a control group (untreated cancer survivors). The LV wall was thinner before GH therapy and increased significantly during 3-years of treatment. GH therapy was also able to increase LV mass, but did not decrease afterload. This beneficial effect was rapidly lost after discontinuation of therapy and, 4 years after, no significant difference was observed between those who received GH and the control group [98]. This study concluded that no evidence of an effective persistent benefit of GH therapy was found. However, the study did not assess whether GH therapy may reduce the global cardiovascular risk in children treated with anthracycline. Indeed, a wide variability of the effects of GH therapy was present: there were patients who did not tolerate GH and developed cardiac failure leading to heart transplantation and other who actually showed a less dramatic cardiac deterioration, no deterioration, or even improvement in cardiac function. Though, many issues remain to be resolved before this approach will be totally dismissed.

It has been reported that GH therapy induces only myocyte hypertrophy and that LV wall thickness and mass are not restored because the number of cardiac myocytes is reduced. However, pig studies suggested that GH treatment results in myocardial growth by increasing capillary density, reducing the rate of apoptosis, and increasing myocardial cell number and size [99]. Additional studies have shown that an

increase in cardiac myocyte number was possible only in young rats, while myocardial cell hypertrophy was the predominant mechanism of GH-induced LV growth in older animals [100, 101].

It could be also possible that the effects of GH on the cardiotoxic heart may involve at least two stem cell populations. One is represented by resident cardiac stem/progenitor cells that possess IGF-1 receptor and upon the action of GH/IGF-1 proliferate and differentiate to replace cardiomyocytes lost as a consequence of cardiotoxic drugs. This hypothesis is strongly supported by the observation that systemic [102] or local [103] IGF-1 activation promotes myocardial regeneration. Intriguingly, cardiac stem cells also express c-met the receptor for Hepatocyte Growth Factor (HGF) and it has also been shown that locally delivered HGF may have beneficial effect on DOX-induced cardiomyopathy [104].

The second potential mechanism implicates a GH mediated proliferation of BM progenitors that, upon peripheral blood mobilization, home to the injured heart where by angiogenetic and differentiative properties may repair anthracycline-induced cardiac damage. The demonstration that GH is able to expand BM stem cells in animals [105] and humans [106] and that circulating progenitors by sensing cardiac injury are recruited at the site of damage [107] strongly favours the possible involvement of BM cells in DOX-induced cardiotoxicity.

Thus, according to these working hypotheses, an increase in LV mass by GH therapy may be explained by the generation of new individual cardiomyocytes derived from BM or resident cardiac stem cell and their reparative properties. More knowledge is needed in order to confirm this new fascinating mechanism potentially exploitable to prevent or attenuate cardiotoxicity by regenerative approaches.

All patients who received chemotherapy at any age, should be educated about the secondary prevention for cardiovascular diseases, since a healthy life-style may be helpful to prevent or decrease cardiotoxicity. Although the influence of a modified healthy diet on cardiotoxicity is limited [58], ideal body weight should be maintained, since obesity is a major preventable coronary risk factor and is related to increased cardiovascular mortality and morbidity. Moreover, additional risk factors, such as diabetes, endocrinopathies, hypertension, or alcohol consumption, drug abuse and cigarettes smoking should be minimized [89].

Aerobic exercise may also be helpful, although patients should be tested to ensure a stable cardiovascular function before starting a physical activity [108]. A 12 weeks, hospital based physical activity program reduces body fat and improves general clinical conditions. Importantly, physical activity may be beneficial in some cancer survivors but harmful in others [108]. Beside these studies, it is our opinion that aerobic exercise should be more critically evaluated for the early prevention of cardiotoxic complications. Indeed, a moderate pre-treatment training program may physiologically induce a better myocardial perfusion and a reduction in afterload, thus positively conditioning the heart, which has to support the subsequent adaptations to repeated drug-mediated cardiac insults. Interestingly, it has also been demonstrated that a long- and short-term voluntary physical exercise up-regulates cardiac telomere-stabilizing proteins [109] and thereby induces antisenescent and protective effects, for example, to prevent doxorubicin-induced cardiomyopathy. These beneficial cardiac effects are mediated by TERT, eNOS, and IGF-1 [109].

TYROSINE KINASE INHIBITORS

Trastuzumab

The ErbB2 (HER2) receptor, a member of the broad family of epidermal growth factor (EGF) receptors (EGFRs), is overexpressed in approximately 25% of human breast cancers. This subset of HER-2–positive breast cancers are associated with advanced clinical stage at presentation, poorer disease-free and overall survival compared to HER-2–negative breast cancers, in the absence of TKIs therapy [110]. This scenario dramatically changed after the approval of Trastuzumab (TTZ), an anti-HER2 humanized monoclonal antibody. TTZ is recommended in metastatic breast cancer as well as in the adjuvant and neoadjuvant therapy of operable breast cancer [111-113]. In all these settings, the addition of TTZ to chemotherapy has led to a remarkable gain in survival.

In the pivotal phase III trial on metastatic setting, the addition of TTZ to DOX and cyclophosphamide or to paclitaxel resulted in prolonged time to progression and improved survival [114]. However, cardiac events were apparent and even monotherapy with TTZ given as second or third line produced cardiac dysfunction as the most significant adverse clinical event [115]. In the pivotal phase II trial of the drug as second- or third-line monotherapy, 6.0% of patients suffered symptomatic heart failure. When follow-up data were included, this rate rose to 8.5%. Further stratification revealed that several patients had not received prior anthracyclines. Retrospective analysis of data from these anthracycline-naive patients indicated that the rate of TTZ-induced heart failure was 3.6% [116].

An independent Cardiac Review and Evaluation Committee (CREC) was held to retrospectively analyse 7 phase II and III clinical trials [117] involving patients treated with TTZ to detect cardiotoxicity which was not apparent in preclinical or in phase I and II clinical studies [69, 118-121]. In this analysis, the administration of anthracyclines and cyclophosphamide (AC) was associated with an 8% rate of overall cardiovascular diseases (CVD) with a New York Heart Association (NYHA) class III/IV rate of 4%. When TTZ was added to AC chemotherapy, the overall CVD rate was 27% with a 16% of patients being in class III/IV of NYHA classification of CHF. Paclitaxel alone was associated with a CVD rate of 1% and NYHA class III/IV rate of 1%. The addition of TTZ to paclitaxel resulted in a 13% rate of CVD with an incidence of NYHA class III/IV patients of 2%. It was the opinion of CREC that the CVD risk associated with the use of TTZ in metastatic setting can be justified, given the 25% improvement in overall survival observed in the pivotal comparative chemotherapy trial and the poor prognosis associated with HER-2–positive breast cancer. In another study, after a median follow-up of 33.6 months and a median time

receiving TTZ of 21.3 months, the overall incidence of cardiac dysfunction was 28% (10.9% NYHA class III) [122]. These positive results in the metastatic setting led to design several trials assessing the efficacy of TTZ in the adjuvant setting for women with HER-2–positive disease.

In general, the adjuvant trials demonstrated that the risk of severe symptomatic CHF was low. However, data revealed that a much greater proportion of patients receiving TTZ experienced low degrees of cardiac dysfunction. Notably, asymptomatic decreases in LVEF were seen in up to 14% of patients in the NSABP B-31 trial [123]. Although many patients recover left ventricular function after discontinuation of TTZ, follow-up cardiac monitoring have suggested that the decline in LVEF was more sustained than previously believed [123].

In the HERA trial, the difference in the rate of cardiac death and severe CHF between Trastuzumab- and nonTrastuzumab-treated patients was less than 4% [124]. Despite this clinical observation, a large number of patients on these trials experienced some form of cardiotoxicity, which ultimately required discontinuation of the drug. Similarly, 14% of patients in the NSABP B-31 trial had TTZ discontinued due to asymptomatic decrease in LVEF.

The reported low incidence of clinically relevant heart failure in patients receiving TTZ is likely to be only the proverbial tip of the iceberg: many cases showing asymptomatic left ventricular dysfunction may well progress to overt heart failure with time, as it has been repeatedly shown after anthracyclines treatment [5]. This is a relevant issue according to the fact that heart failure is known to be a progressive clinical syndrome including several, often silent, evolutive stages. When asymptomatic left ventricular dysfunction progresses to symptomatic heart failure, cardiovascular agents can prolong life, but overt heart failure continues to have a high mortality rate even with modern therapy [125].

Mechanisms of Cardiotoxicity

HER2 is a 185 kDa transmembrane protein with a cytoplasmic tyrosine kinase domain that is normally activated by heterodimerization with HER1, HER3, or HER4. Neuregulins, produced by cardiomyocytes and endothelial cells, cause dimerization of HER2 with HER4, autophosphorylation of the HER2-HER4 heterodimer, increased tyrosine kinase activity and activation of signalling pathways aimed at cell survival and growth [126]. Germ line deletion of HER2 or HER4 or neuregulin-1 disrupts embryonic cardiac development, while cardiac-specific deletion of HER2 late in development causes postnatal dilated cardiomyopathy and reduced cardiac resistance to anthracyclines or other stressor agents [127-130]. More recently, a fundamental role of neuregulins in the control of cardiomyocyte proliferation and heart repair has been clearly documented [131].

In adult patients, TTZ alone causes LV contractile dysfunction that develops independently from the dose and shows reversibility upon medication or drug withdrawal. It has been also shown that LV dysfunction does not relapse upon rechallenge and only occasionally induces ultrastructural damage at endomyocardial biopsies. These observations have represented the basis to distinguish the cardiotoxicity of anthracyclines (type I) from that of TTZ (type II) [132]. On the other hand, TTZ mediated cardiac effects may occur through several alternative mechanisms, including antibody-dependent cell-mediated cytotoxicity (ADCC).

A two-hit model of TTZ-induced cardiotoxicity has been proposed: TTZ first results in loss of the protective effects of ErbB2-mediated signalling which is subsequently translated to alteration in the ability of the heart to respond to stress (for example the interaction with anthracyclines) [133, 134].

The nature and severity of cardiac stress in addition to underlying cardiac risk factors are believed to be important determinants of the magnitude of cardiac injury following TTZ therapy.

Although all these mechanisms, based on experimental and clinical findings, have been proposed, the cellular target of TTZ induced cardiotoxicity remains unknown. Recently, our laboratory has provided evidence of the existence of a resident cardiac progenitor cell population at tissue [37, 38] and cellular [9] levels of organization. Thus, from small myocardial samples of the adult human heart it is possible to isolate and expand multipotent clonogenic cells possessing the *in vitro* and *in vivo* ability to generate all the structural myocardial compartments [9]. We have observed that, in addition to the expression of receptors for several growth factors including c-kit, for Stem Cell Factor (SCF), and c-met, for HGF, these cells express the EGF receptor c-erbB2 (F.Q., personal data). These findings in association with the recent documentation of the essential role of ErbB2 in cardiac repair [131], suggest that TTZ potentially exert its cardiotoxic effect on the human heart by a direct interference on proliferation and survival of primitive cells that control myocardial homeostasis.

Sunitinib and Sorafenib

Sunitinib (SUT) and Sorafenib (SOR) represent examples of the recent trend towards drugs that target multiple receptor kinases by inhibiting Vascular Endothelial Growth Factor (VEGF) receptor 1–3, Platelets Derived Growth Factor (PDGF) receptor α/β, KIT, FMS-related tyrosine kinase 3 (FLT3), colony-stimulating factor 1 receptor (CSF1R) and rearranged during transfection (RET) receptor tyrosine kinases [135].

In a randomized study, patients with previously untreated metastatic renal-cell carcinoma had longer median progression-free survival on SUT (11 months) than those on interferon alfa (5 months), and SUT was also associated with a higher objective response rate (31% *vs.* 6%) [136]. Similarly, in cases of advanced gastrointestinal stromal tumors (GIST) that failed previous treatment with imatinib, the median time to progression in patients receiving SUT was 27 weeks and only 6 weeks in those on placebo. SUT has been approved for the treatment of advanced renal-cell cancer and advanced GIST after resistance or intolerance to imatinib [137].

However, the use of SUT resulted in a decline of ejection fraction [136-138]. Results of three SUT containing trials have indicated contrasting results on LV function. One trial, on patients with advanced GIST, reported no change in

mean LVEF after a median treatment duration of 8 weeks. The other trial, on metastatic renal cell carcinoma, reported that 10% of patients had a decline in LVEF after median treatment duration of 6 months, although in the absence of clinical sequelae. However, LVEF values were not reported.

In an additional study, LVEF declined progressively during SUT from a baseline of 64.5% to 59.4% after the fourth cycle, and 19% of patients had a reduction in LVEF of 15 percentage points or more during treatment. Cardiovascular (fatal or non-fatal) events involved 11% of patients and a large proportion (47%) developed hypertension (>150/100 mmHg). The prescribing information for SUT [139] notes that 11% of patients had a decline in LVEF.

The precise rate of cardiotoxicity associated with TKIs and its reversibility is unknown. Phase III trials have not pursued cardiac end points, and the identification of cardiac adverse effects was predominantly based on the occurrence of clinical symptoms [140]. The analysis of patients treated either with SUT or SOR also suggest that TKI possesses a clinically relevant cardiotoxicity. The high rate of cardiotoxicity in this analysis is related to the definition of a cardiac event. This definition was based on the intention to detect overt as well as early or minor signs of myocardial damage, which, however, may increase with longer treatment periods. The incidence of reduced LVEF of at least 10 percentage points was 14%, but a clear description of those who developed heart failure was not provided [70].

It has been reported that *in vitro* SUT induces prolongation of action potential in Purkinje fibers and *in vivo* QT prolongation in monkey [141]. QT prolongation was observed when drug concentrations were twice the therapeutic dose, corresponding to oral administration of 150 mg. These EKG changes occurred without clinical sequelae. No QT abnormalities were reported by clinical studies employing SUT [140]. Interestingly, one report documented that 2 out of 3 patients developed reversible myocardial ischemia when SOR was given 2 to 3 weeks after failure of SUT treatment [142].

Mechanisms of Cardiotoxicity

A hypothesis on the underlying pathologic mechanisms of cardiotoxicity caused by SUT and SOR has been advanced [135]. Sunitinib-induced inhibition of ribosomal S6 kinase may trigger an intracellular signalling cascade that antagonizes BCL2, a prosurvival factor, and consequently the release of cytochrome c. This effect may induce the activation of the intrinsic apoptotic pathway and ATP depletion on ventricular myocytes leading to left ventricular dysfunction. In addition, SUT mediates inactivation of AMP-activated protein kinase, which is crucial for the cellular response to hypoxia affecting cardiomyocyte survival. Finally, sunitinib-mediated inactivation of AMP-activated protein kinase could promote hypertrophy through increased activity of the eukaryotic elongation factor-2 and mammalian target of rapamycin (mTOR) [135].

For all TKIs described in the present study, alternative pathogenetic mechanisms are suggested in the following paragraph dealing with imatinib mesylate.

Imatinib Mesylate

Imatinib Mesylate (IM) belongs to the family of TKIs, small-molecules with a spectrum of activity that is reasonably selective for certain cell signaling pathways [135]. The inhibited tyrosine kinases by IM include the Abl family encompassing the oncogenic fusion protein product of the leukemogenic Bcr-Abl translocation as well as Abl [143, 144] and the Abl-related gene ARG [145]. In addition, IM possesses a potent activity against mutated oncogenic forms of the receptor tyrosine kinases KIT and PDGFR. This receptor heterogeneity has introduced the use IM in different pathologic conditions like Chronic Myeloid Leukemia (CML), GIST and hypereosinophilic syndrome. Studies are in progress about the use of IM in myelofibrosis with myeloid metaplasia, c-kit positive Acute Myeloid Leukemia, *PDGFRA*–positive eosinophilic disorders (FIP1L-PDFGFR), c-kit positive systemic mastocytosis, policythemia vera, chronic myelomonocytic leukemia (TEL-PDGFR) and dermatofibrosarcoma protuberans (col-PDGF).

The important impact of IM and other drugs of the same class in the oncologic therapy has recently been resized by the finding of cardiotoxicity including symptomatic CHF or asymptomatic LV dysfunction [146].

The initial cases of CHF have been described in IM treated patients lacking previous evidence of cardiomyopathy [146]. Following this observation, many investigators have reported that the incidence of CHF in patients treated with IM might have been overestimated [147]. On the other hand, the magnitude of this phenomenon could be oppositely underestimated because clinical trials have not included predefined cardiac endpoints so that prospective measurement of LV function before and during treatment was not performed [148].

However, due to the ability of TKIs to change the history of some neoplastic diseases, it is imperative to understand the molecular mechanism of action and the cardiotoxic effect of IM [92].

Mechanisms of Cardiotoxicity

Determining the mechanism of toxicity requires the identification of specific targets. The results of such investigations on IM have been surprising, because kinases, like Abl, without a known role in the maintenance of cardiomyocyte "health", have been identified as potential responsible target of cardiotoxicity [146].

It is known that Abl kinase usually mediates apoptosis, so one would not expect cytotoxicity from Abl kinase inhibitors. It has been suggested that cardiotoxicity of IM is mediated by Abl signaling inhibition in cardiomyocytes like it occurs in CML cells, therefore leading to cell death [146]. Indeed, in cardiomyocytes Abl, localized in the plasma membrane and in endoplasmic reticulum (ER), seems to maintain ER homeostasis by unclear mechanisms. IM, dasatinib and nilotinib, by inhibiting Abl, induce ER stress leading to activation of the PKR-like ER kinase (PERK) and IRE1 pathways and to overexpression of protein kinase Cδ (PKCδ). These events activate Jun N-terminal Kinase (JNKs) [149] and the release of BAX followed by mitochon-

drial depolarization, ATP depletion, cytochrome c release, eventually ending in necrotic and apoptotic cell death. In imatinib-resistant mice with c-Abl mutation a marked reduction of cytochrome c release [150] and a protection from imatinib-induced cell death has been also documented *in vitro*.

Left ventricular function and morphologic characteristics of the heart by transmission optical and electron microscopy were analysed in 10 patients under IM therapy [146]. The same evaluation was undertaken in IM treated mice using different concentration of the drug. Patients with normal left ventricular function before IM and followed for 7 months (range 1–14 months) after therapy showed evidence of heart failure, including significant volume overload and symptoms corresponding to a NYHA class III–IV. The low ejection fraction was associated with mild left ventricular dilation. Myocardial biopsies were performed in two of these individuals. Transmission electron micrographs showed prominent membrane "whorls" in myocytes. This abnormality, although unspecific, has been reported to characterize toxin-induced myopathies and is rarely seen in non-ischemic idiopathic dilated cardiomyopathies. Other abnormalities included pleomorphic mitochondria with effaced cristae, scattered cytosolic lipid droplets and vacuoles. In addition, glycogen accumulation in cardiomyocytes was noted [146].

Interestingly, similar findings were observed in mice treated with IM for 3 or 6 weeks at a dose of 50, 100 or 200 mg/kg/die [146]. Transmission electron micrographs from samples of three hearts from treated mice showed numerous membrane "whorls" in the SR and in or immediately adjacent to mitochondria. In addition, SR was substantially dilated. As in human hearts, mitochondrial abnormalities were noted, including evidence of mitochondrial biogenesis—an adaptation typically seen in states of impaired energy generation—with 30% increased number of mitochondria with pleomorphic aspects. Masson's Trichrome staining showed no significant fibrosis. Administration of IM alone to mice, in the absence of co-morbidities, was sufficient to induce cardiotoxicity, culminating in left ventricular contractile dysfunction and dilatation. This is not the case with humans in which the incidence of IM-induced heart failure is relatively low in spite of associated pathologic states frequently present in adult life. Changes in cardiomyocyte dimensions were not significant in the hearts of IM-treated mice. Therefore, the hypothesis has been advanced that myocyte loss was the cause of the reduction in left ventricular mass. Because the TdT mediated dUTP nick end labeling (TUNEL) assay did not suggest increased apoptosis, IM was tested on isolated cardiomyocytes showing a dose dependent collapse of the mitochondrial membrane potential. This was followed by a marked release of cytochrome c into the cytosol and a significant decline in cellular ATP content.

Thus, IM induces abnormalities of mitochondrial morphology and function in the human and mouse heart.

Cellular Targets

Myocyte loss is an important determinant of myocardial dysfunction. Cardiomyocytes constitute 80% of cardiac mass but represent only 20% of cells populating the heart. Thus, other cell types, like fibroblasts, endothelial, smooth muscle or stromal cells are important structural and trophic support of the myocardium. In this regard, fibroblasts and endothelial cells could be sensitive to the toxic insult of anti-cancer drugs in a similar manner as it occurs in contractile cells. It could be also possible that distribution of drugs through extracellular fluids causes a modification of matrix composition and paracrine signals leading to deterioration of cardiomyocytes. Studies supporting this hypothesis are in progress.

The developing and postnatal heart contains multipotent cardiovascular progenitors that can differentiate *in vitro* and *in vivo* in cardiac phenotypes [9, 151]. These cells express c-kit, the receptor for SCF, which is the most preserved SC markers in different tissues of different species [152-157]. Now, the question is whether this cell population could be a target of TKIs acting on KIT ligand and other important molecules regulating SC survival and function. If this is the case, IM may hamper the repairing machinery mediated by c-kit^{pos} myocardial progenitors in response to the cytotoxic effect of IM on cardiomyocytes induced by inhibition on c-Abl.

Thus, preservation of the SC pool may be cardioprotective and the observation that IM interferes with the functional competence and the repairing ability of c-kit^{pos} cells in the heart strongly supports this contention [107].

In order to test this hypothesis we studied the *in vitro* effect of IM on human myocardial cells isolated from the heart [9] and on CML cells taken as a reference. We selected 5 μM concentration of IM because is equivalent to the *in vivo* dose of 400 mg (that is the canonical therapeutic dose) and 50 μM to possibly overcome drug resistance. The amount of genotoxic damage was measured at different time points in cultured cells by the immunocytochemical detection of Histone A2 X positive DNA double strand breaks (DSBs) Fig. (**2A-C**). A dose dependent DNA damage was observed in both cell types 6 hours after IM exposure Fig. (**2 D-E**). Interestingly, lower concentration of IM produced a divergent effect, since in CML cells DSBs formation increased with time whereas a trend to decrease was observed in myocardial progenitors. This phenomenon may be attributed to a possible activation of the DNA repairing machinery allowed only by low dose of IM in myocardial cells and not in Bcr-Abl positive CML [158].

Thus, IM may affect the primitive cell compartment of the human heart blunting the reparative processes implicated in preservation of cardiac integrity.

As for all others drugs under suspicion of cardiotoxicity, also for IM is conceivable that the underlying mechanism should be elucidated in experimental animals. However, it seems that IM is more toxic in mice than in humans, pointing to an important limitation in animal models aimed at predicting drug cardiotoxicity [135].

Risk Factors for TKI$_s$ Cardiotoxicity

Most of the observations on the identification of the risk to develop CVD by TKIs treatment have been made on TTZ. In a pivotal study on metastatic setting [115], increasing age was the only baseline characteristic representing a significant

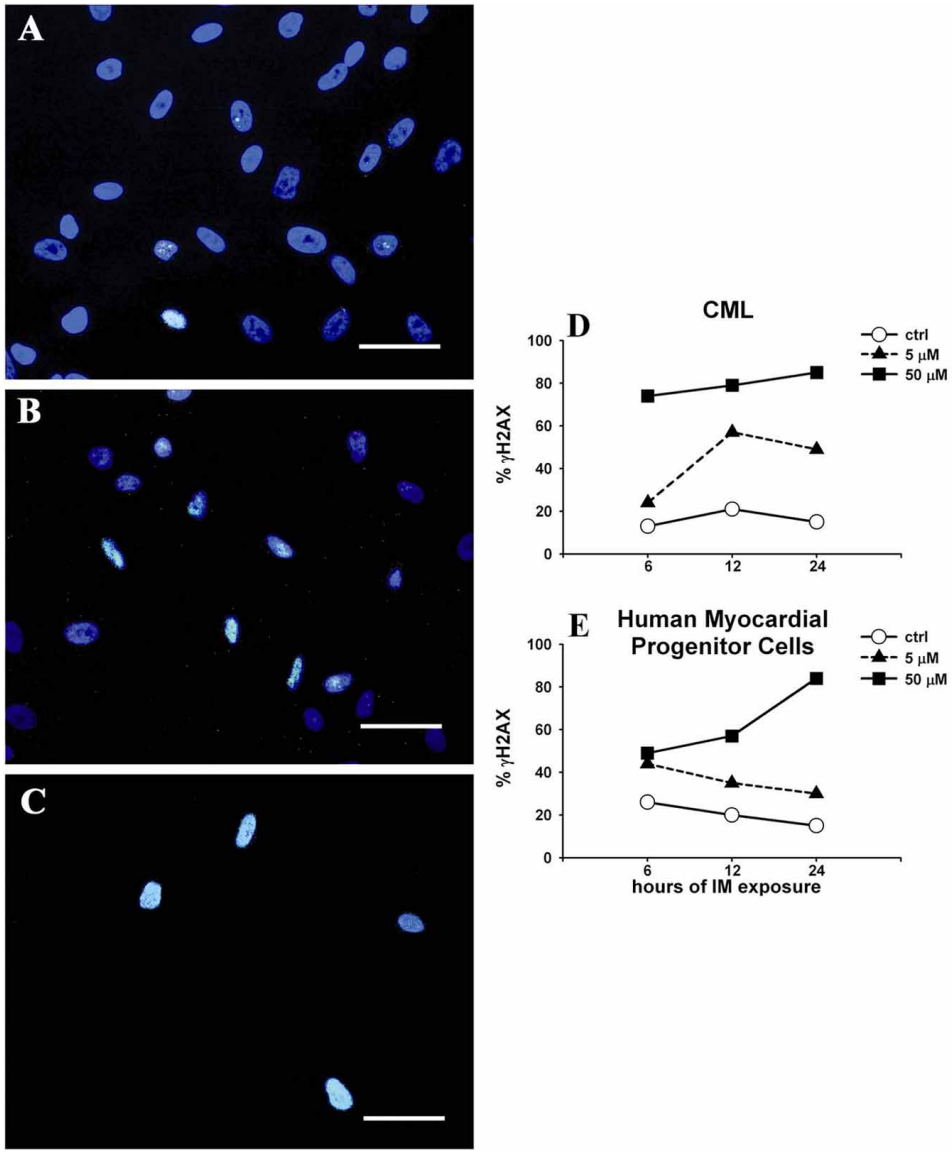

Fig. (2). Imatinib Mesylate (IM) Induces DNA Double Strand Breaks in Human Myocardial Progenitors. **A**-**C**: confocal images of γH2AX labeling (green fluorescence) on nuclei (blue fluorescence) of human myocardial cells cultured in the absence (**A**) and presence of 5 μM (**B**) and 50 μM (**C**) concentrations of IM. Scale bars= 20 μm. D-E: quantification of DNA double strand breaks in Bcr/Abl rearranged BM CML cells (**D**) and myocardial progenitors (**E**) documenting a dose dependent DNA damage at each time point. A time dependent DNA damage was found only with 50 μM concentration of IM on both cell types, whereas 5 μM doses did not exert a linear genotoxic effect with time.

risk factor for cardiac dysfunction in patients receiving the combination of anthracycline, cyclophosphamide and TTZ. However, on multivariate analysis, independent risk factors for the development of cardiac failure included the concomitant use of anthracycline, prior anthracycline exposure, age > 45 years and NYHA class IV before enrolment in the trial [159]. In terms of risk factors for TTZ-associated cardiotoxicity in adjuvant setting**,** in the HERA trial, patients who developed heart failure were treated with higher cumulative doses of DOX or epirubicine and had a lower LVEF and a higher body mass index at screening.

No associations were found between cardiac endpoints and older age, previous cardiac diseases, hyperlipidemia or hypertension. The investigators correctly cautioned that analyses of potential risk factors were exploratory and based on a small number of cardiac events [124]. The 5-year update of the NSABP B-31 identified four risk factors for heart failure in TTZ-treated patients: age, use of hypertensive medications, baseline and post-anthracycline LVEF values [123]. Hypertension, an easily treated and often ignored co-existent condition, is a defined risk factor for the development of heart failure and other cardiovascular events. The incidence of hypertension with TKIs is high and deserves attention early in the course of therapy.

Prevention

Detailed guidelines for the prevention of TKIs mediated cardiotoxicity are lacking. In the attempt to reduce cardiotoxicity of TTZ some hypotheses have been proposed. Increasing the washout period from previous chemotherapy has

been shown to produce positive results in the development of CHF in TTZ treated patients. Ad interim analyses of the HERA trial showed that following 90 days of washout, a lower incidence of symptomatic CHF was observed after 1 year of TTZ treatment [124, 159].

Toxicity of TKIs containing regimens could be prevented by the same attentions used in cardiotoxicity of antracyclines or changing their combination with non-anthracycline regimes (for example paclitaxel and capecytabine) [115, 160-161]. In the FinHer study, in which patients received a short course of TTZ (9 weeks instead of one or two years) before anthracycline-containing regimens, cardiotoxicity was not observed [162].

Monitoring and Follow up

Extensive reports on the principles regulating the monitor and follow-up of TKIs treatment are available only for TTZ. When monitored prospectively, the incidence of TTZ-associated cardiotoxicity appears to be lower than that retrospectively estimated from premarketing data. This may be partly due to over reporting of cardiotoxicity by the pivotal clinical trial and also to vigilant baseline cardiac evaluation adopted in more recent clinical trials [159].

Similarly to anthracycline, ventriculography techniques, such as MUGA scan, have been used to monitor cardiac function. As mentioned earlier, echocardiography may be limited by poor reproducibility and high variability [163-165].

It is recommended that LVEF be re-evaluated every 12 weeks in patients receiving TTZ in the adjuvant setting. Patients who experience cardiac symptoms or a greater than 10% absolute asymptomatic decline in LVEF while receiving TTZ may continue to undergo annual cardiac assessments following completion of the treatment. At this time, no evidence exists to support further cardiac monitoring of patients who have completed TTZ treatment in the absence of cardiac symptoms and no signs of substantial (greater than 10% absolute decrease) asymptomatic LVEF decline [166].

The follow-up data on cardiac function represent a concern and challenge the concept that TTZ-related cardiotoxicity is reversible. Among those patients diagnosed with a cardiac event in NSABP B-31, two thirds continued to receive cardiac medications and 71% had a decrease in LVEF relative to baseline, highlighting persistent cardiac dysfunction. Focusing on severe symptomatic CHF does not tell the whole story. A substantial portion of patients treated with TTZ experience asymptomatic decrements in LVEF (14 % in NSABP B-31 and 17% in BCIRG 006) providing additional evidence supporting a significant persistence of cardiac dysfunction following drug administration [5]. On the other hand, the analysis of cardiac endpoints in the HERA trial suggested that the benefit of TTZ on cancer continues to increase in the second year of follow-up, while the cumulative incidence of any type of cardiac endpoint appears stable after completion of TTZ [124, 159].

A retrospective study showed that symptomatic heart failure occurred soon after initiation of SUT (mean onset 22 days) [167, 168], and was associated with a decline in cardiac function and elevations in blood pressure. These alterations were not completely reversible in most patients even after termination of therapy. The current considerations on the management of IM cardiotoxicity are similar to those described for other TKIs.

It is well known that overt anthracycline cardiotoxicity can become manifest many years after treatment and this may well be the case with TKIs. The risk of such late toxicity might be acceptable for patients with significantly increased overall survival by the agent, but might be unacceptable for others. There is also a general concern that outside the setting of clinical trials, the observed rates of cardiotoxicity may be increased due to less restricted criteria and to more advanced toxicity monitoring. This underscores the importance of patient selection and complies with the cardiotoxicity monitoring strategies used in the adjuvant trials. These observations also highlight the critical importance of continued cardiac follow-up of TKIs treated patients.

These observations suggest that TKIs associated heart failure needs a careful monitoring of cardiac function. Patients with coronary artery diseases, other cardiac pathologies or previous treatment with anthracycline may be at particularly high risk of cardiac failure and possibly myocardial infarction during TKIs therapy, and therefore need very accurate and close follow-up.

Exploratory data also indicate that the early detection of cardiotoxicity associated with TKIs may be predicted by a doubling of BNP levels. [1]

Treatment

Management of TKIs-related cardiotoxicity has two distinct aspects: withdrawal of the drug and treatment of cardiac dysfunction. The “stopping/restarting” rules have been effective and are recommended, with some modifications regarding the need of cardiologic consult or treatment of cardiac dysfunction (or both) when appropriate. Symptomatic left ventricular TTZ-related cardiotoxicity seems to be partially reversible after drug withdrawal and initiation of medical therapy [166, 169].

Pharmacological treatment should be similar to that of patients with LV dysfunction of any other etiology. Most patients in the HERA trial with cardiac dysfunction experienced symptomatic improvement and at least partial recovery of LVEF less than 6 months after TTZ withdrawal and initiation of ACE-I and beta-blockers. Whether TTZ-related LV dysfunction will have a similar natural history or a course modifiable by pharmacologic interventions is unknown. It is likely that many TTZ treated patients were already taking drugs known to favorably impact on the natural history of left ventricular dysfunction. Thus, it is

[1] Kutteh, L.A.; Hobday, T.; Jaffe, A.A correlative study of cardiac biomarkers and left ventricular ejection fraction (LVEF) from N9831, a phase III randomized trial of chemotherapy and trastuzumab as adjuvant therapy for HER2-positive breast cancer. [abstract] *J. Clin. Oncol.,* **2007**, *25*, 579.

possible that the use of these agents may have a beneficial effect on the cumulative incidence of the observed cardiotoxicity [5].

We believe that individualized cardiovascular management is essential for reversibility and additional safety when TKIs are employed. Due to the high incidence of hypertension associated with TKIs treatment, an a priori initiation of ACE-I might consistently ensure blood pressure control that is an important factor to prevent cardiovascular diseases [170, 171].

In addition, it would be important to use drugs that protect myocyte mitochondria, the chief targets for TKIs-mediated cardiotoxicity. However, such an approach has only been demonstrated for IM-induced cardiotoxicity [146]. In the case of other TKIs, co medication with β-blockers, particularly with carvedilol, appears reasonable. Carvedilol, a α/β adrenergic receptor antagonist with antioxidant properties, has a positive impact on cardiac mitochondria and shows beneficial effects on mitochondrial cardiomyopathy [172, 173]. Similarly, statins may protect cardiomyocytes *via* activation of NO synthase and mitochondrial ATP-sensitive potassium channels, and may be considered as possible new candidates with cardioprotective properties in patients treated with TKIs [174].

A specific mention should be made on the recent attempt to produce TKIs molecules with preserved anti-tumor efficacy and reduced toxicity. In this regard, the MD Anderson Cancer Center has been able to reengineer IM by hampering the inhibition of Bcr-Abl, claimed to be responsible for cardiotoxicity, and adding new target kinases to possibly increase antineoplstic activity [175].

SUMMARY

The overall increase population of long-term cancer survivors, together with evidence that, in addition to anthracycline, the recently introduced target therapy may also affect the cardiovascular system, represents an emerging epidemiologic phenomenon requiring urgent measures.

Advanced knowledge of biological and cellular events implicated in the response of the heart to anti-cancer therapy is a fundamental tool for the early detection of cardiotoxicity and for the prevention of cardiovascular events in cancer survivors.

A common subcellular target of anthracycline and TKIs involving mitochondrial dysfunction and damage may explain their preferential toxic effect on the heart, a high energy-consuming organ.

Cardioprotection from anthracycline and TKIs may be achieved by drugs selectively acting on the preservation of ATP-mitochondrial pathways of myocardial cells.

Resident myocardial cell compartments including cardiac progenitors responsible for tissue homeostasis are proposed as new cellular targets of anthracycline and TKIs toxicity.

Approaches aimed at the preservation or expansion of the cardiac progenitor cell pool may change the onset and development of cardiovascular damage by cytotoxic drugs.

CONCLUSION

Epidemiologic studies show an increased incidence and survival of cancer patients. In United States in 2006 the diagnosis of cancer has been reported in 1.5 millions subjects and more than 0.5 millions died of the disease. These impressive data, together with the observation that cancer survival is increased from 50% to 64% in the past 30 years, leads to an estimated number of 10 millions of cancer patients per year. Thus, a constant increase with time of these patients is expected since 70% of tumors can be cured in the pediatric population and 50% of treated patients develop cardiovascular complications. Health organizations and institutions must accomplish a true emergency, which requires scientific knowledge on the basic mechanism, prevention and treatment of cardiovascular events in cancer patients. If our proposed hypotheses are correct the approach to cancer and its social and sanitary impact will inevitably change. Understanding the toxic effects of anti-cancer therapy will improve the clinical benefit of the actual strategy reducing hospitalization and mortality. This type of research implies large-scale studies, a multidisciplinary approach and international networks involving clinical and biologic units.

The big challenges that scientific communities and clinical institutions have to face are currently to find an effective prevention, to discover new beneficial treatments and to avoid or possibly heal cardiac damage caused by anti-cancer strategies.

ABBREVIATIONS

Abl	=	Abelson
ACE-I	=	Angiotensin converting enzyme inhibitor
ADCC	=	Antibody dependent cell mediated cytotoxicity
AHA	=	American Heart Association
AMPK	=	Adenosine monophosphate protein kinase
ARG	=	Abl related genes
ATP	=	Adenosine triphosphate
Bcr	=	Breakpoint cluster region
BM	=	Bone marrow
BNP	=	Brain natriuretic peptide
CHF	=	Congestive heart failure
^{14}C	=	Carbon-14
CAT	=	Computed axial tomography
CK	=	Creatine kinase
CML	=	Chronic myeloid leukemia
CSF	=	Colony stimulating factor
CVD	=	Cardiovascular diseases
DNA	=	Deoxyribonucleic acid
DOX	=	Doxorubicin
DSB	=	Double strand break

dUTP = Deoxyuridine triphosphate

EF = Ejection fraction

EGF = Epidermal growth factor

EKG = Electrocardiogram

eNOS = Endothelial nitric oxide synthase

EPI = Epirubicin

ER = Endoplasmic reticulum

FIP1L = Fip 1 like gene

FLT = FMS-related tyrosine kinase

F.Q. = Federico Quaini

GH = Growth hormone

GIST = Gastrointestinal stromal tumors

Gy = Gray

HDC = High dose chemotherapy

HER-2 = Human epidermal growth factor receptor-2

H&E = Hematoxylin and eosin

HGF = Hepatocyte growth factor

IGF-1 = Insulin-like growth factor-1

IM = Imatinib

JNK = June N-terminal kinase

LV = Left ventricle

mTOR = Mammalian target of rapamycin

MUGA = Multi gated acquisition

NHL = Non-Hodgkin Lymphoma

NO = Nitric oxide

NT = n-terminal

NYHA = New york heart association

PDGF = Platelets derived growth factor

PKC = Protein kinase C

RET = Rearranged during transfection

ROS = Reactive oxygen species

SCF = Stem cell factor

SOR = Sorafenib

SR = Sarcoplasmic reticulum

SUT = Sunitinib

TEL = Translocated ets leukemia

TERT = Telomerase reverse transcriptase

TnI = Troponin I

TKI = Tyrosine kinase inhibitor

TTZ = Trastuzumab

TUNEL = Terminal deoxynucleotidil transferase mediated nick end labeling

VEGF = Vascular endothelial growth factor

REFERENCES

[1] Tilly, H.; Lepage, E.; Coiffier, B.; Blanc, M.; Herbrecht, R.; Bosly, A.; Attal, M.; Fillet, G.; Guettier, C.; Molina, T.J.; Gisselbrecht, C.; Reyes, F. Groupe d'Etude des Lymphomes de l'Adulte. Intensive conventional chemotherapy (ACVBP regimen) compared with standard CHOP for poor-prognosis aggressive non-Hodgkin lymphoma. *Blood*, **2003**, *102*, 4284.

[2] Jones, S.E.; Savin, M.A.; Holmes, F.A. Phase III trial comparing doxorubicin plus cyclophosphamide with docetaxel plus cyclophosphamide as adjuvant therapy for operable breast cancer. *J. Clin. Oncol.*, **2006**, *24*, 5381.

[3] Bonadonna, G.; Monfardini, S.; de Lena, M.; Fossati-Bellani, F. Clinical evaluation of adriamycin, a new antitumour antibiotic. *Br. Med. J.*, **1969**, *3*, 503.

[4] Minotti, G.; Menna, P.; Salvatorelli, E.; Cairo, G.; Gianni, L. Antrayclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacol. Rev.,* **2004**, *56,* 185.

[5] Telli, M.L.; Hunt, S.A.; Carlson, R.W.; Guardino, A.E. Trastuzumab-related cardiotoxicity: calling into question the concept of reversibility. *J. Clin. Oncol.,* **2007**, *25,* 3525.

[6] Gianni, L.; Herman, E.H.; Lipshultz, S.E.; Minotti, G.; Sarvazyan, N.; Sawyer, D. Anthracycline cardiotoxicity: from bench to bedside. *J. Clin. Oncol.,* **2008**, *26,* 3777.

[7] Quaini, F.; Cigola, E.; Lagrasta, C.; Saccani, G.; Quaini, E.; Rossi, C.; Olivetti, G.; Anversa, P. End-stage cardiac failure in humans is coupled with the induction of proliferating cell nuclear antigen and nuclear mitotic division in ventricular myocytes. *Circ. Res.,* **1994**, *75,* 1050.

[8] Bergmann, O.; Bhardwaj, R.D.; Bernard, S.; Zdunek, S.; Barnabé-Heider, F.; Walsh, S.; Zupicich, J.; Alkass, K.; Buchholz, B.A.; Druid, H.; Jovinge, S.; Frisén J. Evidence for cardiomyocyte renewal in humans. *Science,* **2009**, *324*, 98.

[9] Bearzi, C.; Rota, M.; Hosoda, T.; Tillmanns, J.; Nascimbene, A.; De Angelis, A.; Yasuzawa-Amano, S.; Trofimova, I.; Siggins, R.W.; Lecapitaine, N.; Cascapera, S.; Beltrami, A.P.; D'Alessandro, D.A.; Zias, E.; Quaini, F.; Urbanek, K.; Michler, R.E.; Bolli, R.; Kajstura, J.; Leri, A.; Anversa, P. Human cardiac stem cells. *Proc. Natl. Acad. Sci. USA*, **2007**, *104,* 14068.

[10] Oh, H.; Bradfute, S.B.; Gallardo, T.D.; Nakamura, T.; Gaussin, V.; Mishina, Y.; Pocius, J.; Michael, L.H.; Behringer, R.R.; Garry, D.J.; Entman, M.L.; Schneider, M.D. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 12313.

[11] Von Hoff, D.D.; Rozencweig, M.; Layard, M.; Slavik, M.; Muggia, F.M. Daunomicine induced cardiotoxicity in children and adults. A review of 110 cases. *Am. J. Med*., **1977**, *62*, 200.

[12] Carrió, I.; Estorch, M.; Berná, L.; López-Pousa, J.; Tabernero, J.; Torres, G. Indium-111-antimyosin and iodine-123-MIBG studies in early assessment of doxorubicin cardiotoxicity. *J. Nucl. Med*., **1995**, *36*, 2044.

[13] Steinherz, L.J.; Steinherz, P.G.; Tan, C.T.; Heller, G.; Murphy, M.L. Cardiac toxicity 4 to 20 years after completing anthracycline therapy. *JAMA,* **1991**, *266*, 1672.

[14] Pinder, M.C.; Duan, Z.; Goodwin, J.S.; Hortobagyi, G.N.; Giordano, S.H. Congestive heart failure in older women treated with adjuvant anthracycline chemotherapy for breast cancer. *J. Clin. Oncol.,* **2007**, *91*, 37.

[15] Jain, D. Cardiotoxicity of doxorubicin and other anthracycline derivatives. *J. Nucl. Cardiol.,* **2000**, *7,* 53.

[16] Lee, B.H.; Goodenday, L.S.; Muswick, G.J.; Yasnoff, W.A.; Leighton, R.F.; Skeel, R.T. Alterations in left ventricular diastolic function with doxorubicin therapy. *J. Am. Coll. Cardiol*., **1987**, *9,* 184.

[17] Horenstein, M.S.; Vander Heide, R.S.; L'Ecuyer, T.J. Molecular basis of anthracycline-induced cardiotoxicity and its prevention. *Mol. Genet. Metab*., **2000**, *71*, 436.

[18] Swain, S.M.; Whaley, F.S.; Ewer, M.S. Congestive heart failure in patients treated with doxorubicin: A retrospective analysis of three trials. *Cancer,* **2003**, *97,* 2869

[19] Portera, C.C.; Swain, S.M. The heart of the matter. *J. Clin. Oncol.,* **2007**, *25*, 3794.

[20] Church, D.F.; Pryor, W.A. Free-radical chemistry of cigarette smoke and its toxicological implications. *Environ. Health Perspect.,* **1985**, *64*, 111

[21] Prescott, E.; Hippe, M.; Schnohr, P.; Hein, H.O.; Vestbo, J. Smoking and risk of myocardial infarction in women and men: longitudinal population study. *Br. Med. J.,* **1998**, *316*, 1043

[22] Wilson, J.R.; Schwartz, J.S.; Sutton, M.S.; Ferraro, N.; Horowitz, L.N.; Reichek, N.; Josephson, M.E. Prognosis in severe heart failure: relation to hemodynamic measurements and ventricular ectopic activity. *J. Am. Coll. Cardiol.,* **1983**, *2,* 403

[23] Smith, W.M. Epidemiology of congestive heart failure. *Am. J. Cardiol.*, **1985**, *55*, 3.

[24] McKee, P.A.; Castelli, W.P.; McNamara, P.M.; Kannel, W.B. The natural history of congestive heart failure: the Framingham study. *N. Engl. J. Med.,* **1971**, *285*, 1441.

[25] Glader, E.L.; Stegmayr, B.; Norrving, B.; Terent, A.; Hulter-Asberg, K.; Wester, P.O.; Asplund, K. Sex differences in management and outcome after stroke: a Swedish national perspective. *Stroke,* **2003**, *34*, 1970.

[26] Muss, H.B.; Woolf, S.H.; Cirrincione, C.; Weiss, R.B.; Budman, D.; Wood, W.C.; Henderson, I.C.; Hudis, C.; Winer, E.; Cohen, H.; Wheeler, J.; Norton, L. Cancer and Leukemia Group B. Adjuvant chemotherapy in older and younger women with lymph node-positive breast cancer. *JAMA,* **2005**, *293*, 1073.

[27] Doyle, J.J.; Neugut, A.I.; Jacobson, J.S.; Grann, V.R.; Hershman, D.L. Chemotherapy and cardiotoxicity in older breast cancer patients: a population-based study. *J. Clin. Oncol.,* **2005***, 23,* 8597.

[28] Zambetti, M.; Moliterni, A.; Materazzo, C.; Stefanelli, M.; Cipriani, S.; Valagussa, P.; Bonadonna, G.; Gianni, L. Long term cardiac sequelae in operable breast cancer patients given adjuvant chemotherapy with or without doxorubicin and breast irradiation. *J. Clin. Oncol.,* **2001**, *19*, 37.

[29] Sawyer, D.B.; Zuppinger, C,; Miller, T.A.; Suter, T.M. Modulation of anthracycline-induced myofibrillar disarray in rat ventricular myocytes by neuregulin-1beta and anti-erbB2: potential mechanism for trastuzumab-induced cardiotoxicity. *Circulation,* **2002**, *105*, 1551.

[30] Ladas, E.J.; Jacobson, J.S.; Kennedy, D.D. Antioxidants and cancer therapy: a systematic review. *J. Clin. Oncol.,* **2004**, *22,* 517.

[31] Kalay, N.; Basar, E.; Ozdogru, I.; Er, O.; Cetinkaya, Y.; Dogan, A.; Inanc, T.; Oguzhan, A.; Eryol, N.K.; Topsakal, R.; Ergin, A. Protective effects of carvedilol against anthracycline-induced cardiomyopathy. *J. Am. Coll. Cardiol.,* **2006**, *48,* 2258.

[32] Santos, D.L.; Moreno, A.J.; Leino, R.L.; Froberg, M.K.; Wallace, K.B. Carvedilol protects against doxorubicin-induced mitochondrial cardiomyopathy. *Toxicol. Appl. Pharmacol.*, **2002**, *185*, 218.

[33] Lipshultz, S.E.; Colan, S.D.; Gelber, R.D. Late cardiac effects of doxorubicin therapy for acute lymphoblastic leukemia in childhood. *N. Engl. J. Med.,* **1991**, *324*, 808.

[34] Swain, S.M.; Whaley, F.S.; Gerber, M.C.; Weisberg, S.; York, M.; Spider, D.; Jones, S.E.; Wadler, S.; Desai, A.; Vogel, C.; Speyer, J.; Mittelman, A.; Reddy, S.; Pendergrass, K.; Velez-Garcia, E.; Ewer, M.S; Bianchine, J.R.; Gams, R.A. Cardioprotection with dexrazoxane for doxorubicin-containing therapy in advanced breast cancer. *J. Clin. Oncol.,* **1997**, *15*, 1318.

[35] Swain, S.M.; Vici, P. The current and future role of dexrazoxane as a cardioprotectant in anthracycline treatment: export panel review. *J. Cancer Res. Clin. Oncol.,* **2004***, 130*, 1.

[36] Singal, P.K.; Iliskovic, N.; Li, T.; Kumar, D. Adriamycin cardiomyopathy: pathophysiology and prevention. *FASEB J*., **1997**, *11*, 931.

[37] Urbanek, K.; Quaini, F.; Tasca, G.; Torella, D.; Castaldo, C.; Nadal-Ginard, B.; Leri, A.; Kajstura, J.; Quaini, E.; Anversa, P. Intense myocyte formation from cardiac stem cells in human cardiac hypertrophy. *Proc. Natl. Acad. Sci. USA,* **2003**, *100,* 10440.

[38] Urbanek, K.; Torella, D.; Sheikh, F.; De Angelis, A.; Nurzynska, D.; Silvestri, F.; Beltrami, C.A.; Bussani, R.; Beltrami, A.P.; Quaini, F.; Bolli, R.; Leri, A.; Kajstura, J.; Anversa, P. Myocardial regeneration by activation of multipotent cardiac stem cells in ischemic heart failure. *Proc. Natl. Acad. Sci. USA,* **2005**, *102*, 8692.

[39] Olivetti, G.; Abbi, R.; Quaini, F.; Kajstura, J.; Cheng, W.; Nitahara, J.A.; Quaini, E.; Di Loreto, C.; Beltrami, C.A.; Krajewsky, S.; Reed, J.; Anversa, P. Apoptosis in the failing human heart. *N. Engl. J. Med.,* **1997**, *336*, 1131.

[40] Kajstura, J.; Cheng, W.; Sarangarajan, R.; Li, P.; Li, B.; Nitahara, J.A.; Chapnick, S.; Reiss, K.; Olivetti, G.; Anversa, P. Necrotic and apoptotic myocyte cell death in the aging heart of Fischer 344 rats. *Am. J. Physiol*., **1996**, *271*, H1215.

[41] Guerra, S.; Leri, A.; Wang, X.; Finato, N.; Di Loreto, C.; Beltrami, C.A; Kajstura, J.; Anversa, P. Myocyte death in the failing human heart is gender dependent. *Circ. Res.*, **1999**, *85*, 856.

[42] Amir, G.; Ma, X.; Mohan Reddy, V.; Hanley, F.L.; Reinhartz, O.; Ramamoorthy, C.; Riemer, R.K. Dynamics of human myocardial progenitor cell populations in the neonatal period. *Ann. Thorac. Surg*, **2008**, *86*, 1311.

[43] Tokarska-Schlattner, M.; Wallimann, T.; Schlattner, U. Alterations in myocardial energy metabolism induced by the anti-cancer drug doxorubicin. *C. R. Biol.,* **2006**, *329*, 657.

[44] Nicolay, K.; Fok J.J.; Voorhout, W.; Post, J.A.; de Kruijff, B. Cytofluorescence detection of adriamycin-mitochondria interactions in isolated, perfused rat heart. *Biochim. Biophys. Acta*, **1986**, *887*, 35.

[45] Chen, B.; Peng, X.; Pentassuglia, L.; Lim, C.C.; Sawyer, D.B. Molecular and cellular mechanisms of anthracycline cardiotoxicity. *Cardiovasc. Toxicol.,* **2007**, *7*, 114.

[46] Olson, R.D.; Mushlin, P.S. Doxorubicin cardiotoxicity: analysis of prevailing hypotheses. *FASEB J*., **1990**, *4*, 3076.

[47] Singal, P.K.; Iliskovic, N. Doxorubicin-induced cardiomyopathy. *N. Engl. J. Med.,* **1998,** *339*, 900.

[48] Lipshultz, S.E.; Grenier, M.A.; Colan, S.D. Doxorubicin-induced cardiomyopathy. *N. Engl. J. Med.,* **1999**, *340*, 653; author reply, 655.

[49] Menaschè, P. Cell-based Therapy for Heart Disease: A Clinically Oriented Perspective. *Mol. Ther.,* **2009**, *175*, 758.

[50] De Angelis, A.; Piegari, E.; Cappetta, D.; Marino, L.; Filippelli, A.; Berrino, L.; Ferreira-Martins, J.; Zheng, H.; Hosoda, T.; Rota, M.; Urbanek, K.; Kajstura, J.; Leri, A.; Rossi, F.; Anversa, P. Anthracycline cardiomyopathy is mediated by depletion of the cardiac stem cell pool and is rescued by restoration of progenitor cell function. *Circulation*, **2009**, Dec 28. [Epub ahead of print].

[51] Alvarez, J.; Scully, R.; Miller, T. Long-term effects of treatments for childhood cancers. *Curr. Opin. Pediatr.,* **2007**, *19,* 23.

[52] Oeffinger, K.C.; Mertens, A.C.; Sklar, C.A. Chronic health conditions in adult survivors of childhood cancer. *N. Engl. J. Med.,* **2006**, *355*, 1572.

[53] Kremer, L.C.; van Dalen, E.C.; Offringa, M. Anthracycline-induced clinical heart failure in a cohort of 607 children: Long-term follow-up study. *J. Clin. Oncol.,* **2001**, *19*, 191.

[54] Grenier, M.A.; Lipshultz, S.E. Epidemiology of anthracycline cardiotoxicity in children and adults. *Semin. Oncol.*, **1998**, *25,* 72

[55] Kremer, L.C.; van der Pal, H.J.; Offringa, M. Frequency and risk factors of subclinical cardiotoxicity after anthracycline therapy in children: a systematic review. *Ann. Oncol.,* **2002,** *13*, 819.

[56] Barry, E.; Alvarez, J.A.; Scully, R.E. Anthracycline-induced cardiotoxicity: course, pathophysiology, prevention and management. *Expert Opin. Pharmacother.,* **2007**, *8*, 1039.

[57] Krischer, J.P.; Epstein, S.; Cuthbertson, D.D.; Goorin, A.M.; Epstein, M.L.; Lipshultz, S.E. Clinical cardiotoxicity following anthracycline treatment for childhood cancer: the Pediatric Oncology Group experience. *J. Clin. Oncol.,* **1997**, *15*, 1544.

[58] Simbre, V.C.; Duffy, S.A.; Dadlani, G.H.; Miller T.L.; Lipshultz S.E. Cardiotoxicity of cancer chemotherapy: implications for children. *Paediatr. Drugs*, **2005**, *7*, 187.

[59] Pein, F.; Sakiroglu, O.; Dahan, M.; Lebidois, J.; Merlet, P.; Shamsaldin, A.; Villain, E.; de Vathaire, F.; Sidi, D.; Hartmann, O. Cardiac abnormalities 15 years and more after adriamycin therapy in 229 childhood survivors of a solid tumour at the Institut Gustave Roussy. *Br. J. Cancer,* **2004**, *91,* 37.

[60] Lipshultz, S.E.; Lipsitz, S.R.; Mone, S.M. Female sex and drug dose as risk factors for late cardiotoxic effects of doxorubicin therapy for childhood cancer. *N. Engl. J. Med.*, **1995**, *332*, 1738.

[61] Lipshultz, S.E.; Lipsitz, S.R.; Sallan, S.E. Chronic progressive cardiac dysfunction years after doxorubicin therapy for childhood acute lymphoblastic leukemia. *J. Clin. Oncol.,* **2005**, *23*, 2629.

[62] Lipshultz, S.E.; Alvarez, J.A.; Scully, R.E. Anthracycline associated cardiotoxicity in survivors of childhood cancer *Heart,* **2008**, *94*, 525.

[63] Adams, M.J.; Lipshultz, S.E. Pathophysiology of anthracycline- and radiation-associated cardiomyopathies: implications for screening and prevention. *Pediatr. Blood Cancer*, **2005**, *44*, 600.

[64] Lee, Y.T.; Chan, K.K.; Harris, P.A. Tissue disposition of doxorubicin in experimental animals. *Med. Pediatr. Oncol.*, **1982**, *10*, 259.

[65] Rodvold, K.A.; Rushing, D.A.; Tewksbury, D.A. Doxorubicin clearance in the obese. *J. Clin. Oncol.,* **1988**, *6*, 1321.

[66] Olivetti, G.; Giordano, G.; Corradi, D.; Melissari, M.; Lagrasta, C.; Gambert, S.R,; Anversa, P. *J.* Gender differences and aging: Effects on the human heart. *Am. Coll. Cardiol.,* **1995**, *26*, 1068.

[67] Leri, A.; Kajstura, J.; Li, B.; Sonnenblick, E.H.; Beltrami, C.A.; Anversa, P.; Frishman, W.H. Cardiomyocyte aging is gender-dependent: the local IGF-1-IGF-1R system. *Heart Dis*., **2000**, *2*, 108.

[68] Anversa, P.; Palackal, T.; Sonnenblick, E.H.; Olivetti, G.; Meggs, L.G.; Capasso, J.M. Myocyte cell loss and myocyte cellular hyperplasia in the hypertrophied aging rat heart. *Circ. Res.,* **1990**, *67*, 871.

[69] Pai, V.B.; Nahata, M.C. Cardiotoxicity of chemotherapeutic agents. Incidence, treatment and prevention. *Drug Saf.*, **2000**, *22*, 263.

[70] Ewer, M.S.; Lenihan, D.J. Left ventricular ejection fraction and cardiotoxicity: is our ear really to the ground? *J. Clin. Oncol.,* **2008**, *26*, 1201.

[71] Ritchie, J.L.; Bateman, T.M.; Bonow, R.O.; Crawford, M.H.; Gibbons, R.J.; Hall, R.J.; O'Rourke, R.A.; Parisi, A.F.; Verani, M.S. Guidelines for clinical use of cardiac radionuclide imaging. Report of the American College of Cardiology/American Heart Association Task Force on Assessment of Diagnostic and Therapeutic Cardiovascular Procedures (Committee on Radionuclide Imaging), developed in collaboration with the American Society of Nuclear Cardiology. *J. Am. Coll. Cardiol.,* **1995**, *25*, 521.

[72] Jensen, B.V.; Skovsgaard, T.; Nielsen, S.L. Functional monitoring of anthracycline cardiotoxicity: a prospective blinded long term observational study of outcome in 120 patients. *Ann. Oncol.,* **2002**, *13*, 699.

[73] Adamcova, M.; Simunek, T.; Kaiserova, H. *In vitro* and *in vivo* examination of cardiac troponins as biochemical markers of drug-induced cardiotoxicity. *Toxicology*, **2007**, *237*, 218.

[74] Piegari, E.; Di Salvo, G.; Castaldi, B.; Vitelli, M.R.; Rodolico, G.; Golino, P.; Calabrò, R.; Rossi, F.; Berrino, L. Myocardial strain analysis in a doxorubicin-induced cardiomyopathy model. *Ultrasound Med. Biol.,* **2008**, *34*, 370.

[75] Zuppinger, C.; Timolati, F.; Suter, T.M. Pathophysiology and diagnosis of cancer drug induced cardiomyopathy. *Cardiovasc. Toxicol.,* **2007**, *7*, 61.

[76] Tassan-Mangina, S.; Codorean, D.; Metivier, M.; Costa, B.; Himberlin, C.; Jouannaud, C.; Blaise, A.M.; Elaerts, J.; Nazeyrollas, P. Tissue Doppler imaging and conventional echocardiography after anthracycline treatment in adults: early and late alterations of left ventricular function during a prospective study. *Eur. J. Echocardiogr.,* **2006**, *7*, 141.

[77] Yu, C.M.; Sanderson, J.E.; Marwick, T.H.; Oh, J.K. Tissue doppler imaging a new prognosticator for cardiovascular diseases. *J. Am. Coll. Cardiol.,* **2007**, *49*, 1903.

[78] Lipshultz, S.E.; Sanders, S.P.; Goorin, A.M. Monitoring for anthracycline cardiotoxicity. *Pediatrics*, **1994**, *93*, 433.

[79] Carver, J.R.; Schuster, S.J.; Glick, J.H. Doxorubicin cardiotoxicity in the elderly: old drugs and new opportunities. *J. Clin. Oncol.,* **2008**, *26*, 3122.

[80] Cardinale, D.; Sandri, M.T.; Martinoni, A.; Borghini, E.; Civelli, M.; Lamantia, G.; Cinieri, S.; Martinelli, G.; Fiorentini, C.; Cipolla, C.M. Myocardial injury revealed by plasma troponin I in breast cancer treated with high dose chemotherapy. *Ann. Oncol.,* **2002**, *13*, 710.

[81] Meinardi, M.T.; van Veldhuisen, D.J.; Gietema, J.A.; Dolsma, W.V.; Boomsma, F.; van den Berg, M.P.; Volkers, C.; Haaksma, J.; de Vries, E.G.; Sleijfer, D.T.; van der Graaf, W.T. Prospective evaluation of early cardiac damage induced by epirubicin containing adjuvant chemotherapy and locoregional radiotherapy in breast cancer patients. *J. Clin. Oncol.,* **2001**, *19*, 2764.

[82] Ekstein, S.; Nir, A.; Rein, A.J.; Perles, Z.; Bar-Oz, B.; Salpeter, L.; Algur, N.; Weintraub, M. Weintraub M. N-terminal-proB-type natriuretic peptide as a marker for acute anthracycline cardiotoxicity in children. *J. Pediatr. Hematol. Oncol*., **2007**, *29*, 440.

[83] Mavinkurve-Groothuis, A.M.; Groot-Loonen, J.; Bellersen, L.; Pourier, M.S.; Feuth, T.; Bökkerink, J.P.; Hoogerbrugge, P.M.; Kapusta, L. Abnormal NT-pro-BNP levels in asymptomatic long-term survivors of childhood cancer treated with anthracyclines. *Pediatr. Blood Cancer*., **2009**, *52*, 631.

[84] Lipshultz, S.E.; Giantris, A.L.; Lipsitz, S.R. Doxorubicin administration by continuous infusion is not cardioprotective: the Dana-Farber 91-01 acute lymphoblastic leukemia protocol. *J. Clin. Oncol.,* **2002**, *20*, 1677.

[85] Cardinale, D.; Colombo, A.; Sandri, M.T. Prevention of high-dose chemotherapy-induced cardiotoxicity in high-risk patients by angiotensin-converting enzyme inhibition. *Circulation,* **2006**, *114*, 2474.

[86] Nakamae, H.; Tsumura, K.; Terada, Y.; Nakane, T.; Nakamae, M.; Ohta, K.; Yamane, T.; Hino, M. Notable effects of angiotensin II receptor blocker, valsartan, on acute cardiotoxic changes after standard chemotherapy with cyclophosphamide, doxorubicin, vincristine, and prednisolone. *Cancer,* **2005,** *104*, 2492.

[87] Lipshultz, S.E.; Colan, S.D. Cardiovascular trials in long-term survivors of childhood cancer. *J. Clin. Oncol.,* **2004**, *22,* 769.

[88] Lipshultz, S.E.; Lipsitz, S.R.; Sallan, S.E.; Simbre, V.C. 2nd.; Shaikh, S.L.; Mone, S.M.; Gelber, R.D.; Colan, S.D. Long-term enalapril therapy for left ventricular dysfunction in doxorubicin-treated survivors of childhood cancer. *J. Clin. Oncol.,* **2002**, *20*, 4517.

[89] Carver, J.R.; Shapiro, C.L.; Ng, A.; Jacobs, L.; Schwartz, C.; Virgo, K.S.; Hagerty, K.L.; Somerfield, M.R.; Vaughn, D.J. American Society of Clinical Oncology clinical evidence review on the ongoing care of adult cancer survivors: cardiac and pulmonary late effects. *J. Clin. Oncol.,* **2007**, *25,* 3991.

[90] Silber, J.H.; Cnaan, A.; Clark, B.J.; Paridon, S.M.; Chin, A.J.; Rychik, J.; Hogarty, A.N.; Cohen, M.I.; Barber, G.; Rutkowski, M.; Kimball, T.R.; Delaat, C.; Steinherz, L.J.; Zhao, H. Enalapril to prevent cardiac function decline in long-term survivors of pediatric cancer exposed to anthracyclines. *J. Clin. Oncol.,* **2004**, *22,* 820.

[91] Lipshultz, S.E.; Rifai, N.; Dalton, V.M.; Levy, D.E.; Silverman, L.B.; Lipsitz, S.R.; Colan, S.D.; Asselin, B.L.; Barr, R.D.; Clavell, L.A.; Hurwitz, C.A.; Moghrabi, A.; Samson, Y.; Schorin, M.A.; Gelber, R.D.; Sallan, S.E. The effect of dexrazoxane on myocardial injury in doxorubicin-treated children with acute lymphoblastic leukemia. *N. Engl. J. Med.,* **2004**, *351*, 145.

[92] Menna, P.; Salvatorelli, E.; Minotti, G. Cardiotoxicity of antitumor drugs. *Chem. Res. Toxicol*., **2008**, *21*, 978.

[93] Hershman, D.L.; Shao, T. Anthracycline cardiotoxicity after breast cancer treatment. *Oncology*, *(Williston Park)*, **2009**, *23,* 227.

[94] Gabizon, A.A. Liposomal anthracyclines. *Hematol. Oncol. Clin. North Am.*, **1994**, *8*, 431.

[95] O'Brien, M.E.; Wigler, N.; Inbar, M.; Rosso, R.; Grischke, E.; Santoro, A.; Catane, R.; Kieback, D.G.; Tomczak, P.; Ackland, S.P.; Orlandi, F.; Mellars, L.; Alland, L.; Tendler, C. Reduced cardiotoxicity and comparable efficacy in a phase III trial of pegylated liposomal doxorubicin HCl (CAELYX/Doxil) versus conventional doxorubicin for first-line treatment of metastatic breast cancer. *Ann. Oncol.*, **2004**, *15*, 440.

[96] Santucci, L.; Mencarelli, A.; Renga, B.; Ceccobelli, D.; Pasut, G.; Veronese, F.M.; Distrutti, E.; Fiorucci, S. Cardiac safety and antitumoral activity of a new nitric oxide derivative of pegylated epirubicin in mice. *Anticancer Drugs.*, **2007**, *18*, 1081.

[97] Kratz, F. DOXO-EMCH (INNO-206): the first albumin-binding prodrug of doxorubicin to enter clinical trial. *Expert Opin. Invest. Drugs*, **2007**, *16*, 855.

[98] Lipshultz, S.E.; Vlach, S.A.; Lipsitz, S.R. Cardiac changes associated with growth hormone therapy among children treated with anthracyclines. *Pediatrics,* **2005**, *115*, 1613.

[99] Houck, W.V.; Pan, L.C.; Kribbs, S.B.; Clair, M.J.; McDaniel, G.M.; Krombach, R.S.; Merritt, W.M.; Pirie, C.; Iannini, J.P.; Mukherjee, R.; Spinale, F.G. Effects of growth hormone supplementation on left ventricular morphology and myocyte function with development of congestive heart failure. *Circulation,* **1999**, *100*, 2003.

[100] Bruel, A.; Oxlund, H.; Nyengaard, J.R. Growth hormone increases the total number of myocyte nuclei in the left ventricle of adult rats. *Growth Horm. IGF Res.,* **2002**, *12*, 106.

[101] Bruel, A.; Oxlund, H.; Nyengaard, J.R. Growth hormone increases the total number of cardiac myocyte nuclei in young rats but not in old rats. *Mech. Ageing Dev.,* **2002**, *123*, 1353.

[102] Li, Q.; Wu, S.; Li, S.Y.; Lopez, F.L.; Du, M.; Kajstura, J.; Anversa, P.; Ren, J. Cardiac-specific overexpression of insulin-like growth factor 1 attenuates aging-associated cardiac diastolic contractile dysfunction and protein damage. *Am. J. Physiol. Heart Circ. Physiol*., **2007**, *292*, H1398.

[103] Urbanek, K.; Rota, M.; Cascapera, S.; Bearzi, C.; Nascimbene, A.; De Angelis, A.; Hosoda, T.; Chimenti, S.; Baker, M.; Limana, F.; Nurzynska, D.; Torella, D.; Rotatori, F.; Rastaldo, R.; Musso, E.; Quaini, F.; Leri, A.; Kajstura, J.; Anversa, P. Cardiac stem cells possess growth factor-receptor systems that after activation regenerate the infarcted myocardium, improving ventricular function and long-term survival. *Circ. Res*., **2005,** *97*, 663. Erratum in: *Circ. Res.,* **2006**, *98*, e27.

[104] Iwasaki, M.; Adachi, Y.; Nishiue, T.; Minamino, K.; Suzuki, Y.; Zhang, Y.; Nakano, K.; Koike, Y.; Wang, J.; Mukaide, H.; Taketani, S.; Yuasa, F.; Tsubouchi, H.; Gohda, E.; Iwasaka, T.; Ikehara, S. Hepatocyte growth factor delivered by ultrasound-mediated destruction of microbubbles induces proliferation of cardiomyocytes and amelioration of left ventricular contractile function in doxorubicin-induced cardiomyopathy. *Stem Cells,* **2005**, *23*, 1589.

[105] Carlo-Stella, C.; Di Nicola, M.; Milani, R.; Longoni, P.; Milanesi, M.; Bifulco, C.; Stucchi, C.; Guidetti, A.; Cleris, L.; Formelli, F.; Garotta, G.; Gianni, A.M. Age- and irradiation-associated loss of bone marrow hematopoietic function in mice is reversed by recombinant human growth hormone. *Exp. Hematol.*, **2004***, 32*, 171.

[106] Carlo-Stella, C.; Di Nicola, M.; Milani, R.; Guidetti, A.; Magni, M.; Milanesi, M.; Longoni, P.; Matteucci, P.; Formelli, F.; Ravagnani, F.; Corradini, P.; Gianni, A.M. Use of recombinant human growth hormone (rhGH) plus recombinant human granulocyte colony-stimulating factor (rhG-CSF) for the mobilization and collection of CD34+ Cells in Poor Mobilizers. *Blood*, **2004**, *103,* 3287.

[107] Fazel, S.; Cimini, M.; Chen, L.; Li, S.; Angoulvant, D.; Fedak, P.; Verma, S.; Weisel, R.D.; Keating, A.; Li, R.K. Cardioprotective c-kit+ cells are from the bone marrow and regulate the myocardial balance of angiogenic cytokines. *J. Clin. Invest.,* **2006**, *116*, 1865.

[108] Miller, T.L.; Horgan, S.; Lipshultz S.E. Exercise rehabilitation of pediatric patients with cardiovascular disease. *Prog. Pediatr. Cardiol.,* **2005**, *20*, 27.

[109] Werner, C.; Hanhoun, M.; Widmann, T.; Kazakov, A.; Semenov, A.; Pöss, J.; Bauersachs, J.; Thum, T.; Pfreundschuh, M.; Müller, P.; Haendeler, J.; Böhm, M.; Laufs, U. Effects of physical exercise on myocardial telomere-regulating proteins, survival pathways, and apoptosis. *J. Am. Coll. Cardiol.*, **2008**, *52*, 470.

[110] Slamon, D.J.; Clark, G.M.; Wong, S.G.; Levin, W.J.; Ullrich, A.; McGuire, W.L. Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science,* **1987**, *235*, 177.

[111] Buzdar, A.U.; Ibrahim, N.K.; Francis, D.; Booser, D.J.; Thomas, E.S.; Theriault, R.L.; Pusztai, L.; Green, M.C.; Arun, B.K.; Giordano, S.H.; Cristofanilli, M.; Frye, D.K.; Smith, T.L.; Hunt, K.K.; Singletary, S.E.; Sahin, A.A.; Ewer, M.S.; Buchholz, T.A.; Berry, D.; Hortobagyi, G.N. Significantly higher pathologic complete remission rate after neoadjuvant therapy with trastuzumab, paclitaxel, and epirubicin chemotherapy: results of a randomized trial in human epidermal growth factor receptor 2-positive operable breast cancer. *J. Clin. Oncol.,* **2005**, *16*, 3676.

[112] Buzdar, A.U.; Valero, V.; Ibrahim, N.K.; Francis, D.; Broglio, K.R.; Theriault, R.L.; Pusztai, L.; Green, M.C.; Singletary, S.E.; Hunt, K.K.; Sahin, A.A., Esteva, F.; Symmans, W.F.; Ewer, M.S.; Buchholz, T.A.; Hortobagyi, G.N. Neoadjuvant therapy with paclitaxel followed by 5-fluorouracil, epirubicin, and cyclophosphamide chemotherapy and concurrent trastuzumab in human epidermal growth factor receptor 2-positive operable breast cancer: an update of the initial randomized study population and data of additional patients treated with the same regimen. *Clin. Cancer Res.,* **2007**, *1*, 228.

[113] Gianni, L.; Eiermann, W.; Pusztai, L. Biomarkers as potential predictors of pathologic complete response (pCR) in the NOAH trial of neoadjuvant trastuzumab in patients (pts) with HER2-positive locally advanced breast cancer (LABC). *J. Clin. Oncol.,* **2008**, *26*, 504.

[114] Slamon, D.J.; Leyland-Jones, B.; Shak, S. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med.,* **2001**, *344*, 783.

[115] Cobleigh, M.A.; Vogel, C.L.; Tripathy, D. Multinational study of the efficacy and safety of humanised anti-HER2 cardiotoxicity associated with trastuzumab in MBC 181 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J. Clin. Oncol.,* **1999**, *17*, 2639.

[116] Vogel, C.; Cobleigh, M.; Tripathy, D. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J. Clin. Oncol.,* **2002**, *20*, 719.

[117] Seidman, A.; Hudis, C.; Pierri, M.K.; Shak, S.; Paton, V.; Ashby, M.; Murphy, M.; Stewart, S.J.; Keefe, D. Cardiac dysfunction in the trastuzumab clinical trials experience. *J. Clin. Oncol.*, **2002**, *20*, 1215.

[118] Carter, P.; Presta, L.; Gorman, C.M. Humanization of an anti-p185HER2 antibody for human cancer therapy. *Proc. Natl. Acad. Sci. USA*, **1992**, *89*, 4285.

[119] Baselga, J.; Tripathy, D.; Mendelsohn, J. Phase II study of weakly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu overexpressing metastatic breast cancer. *J. Clin. Oncol.,* **1996**, *14,* 737.

[120] Pegram, M.D.; Lipton, A.; Hayes, D.F.; Weber, B.L.; Baselga, J.M.; Tripathy, D.; Baly, D.; Baughman, S.A.; Twaddell, T.; Glaspy, J.A.; Slamon, D.J. Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu overexpressing metastatic breast cancer refractory to chemotherapy treatment. *J. Clin. Oncol.,* **1998**, *16*, 2659.

[121] Shak, S. Herceptin Multinational Investigator Study Group overview of the trastuzumab (Herceptin) anti-HER2 monoclonal antibody clinical program in HER2-overexpressing metastatic breast cancer. *Semin. Oncol.*, **1999**, *26*, 71.

[122] Guarneri, V.; Lenihan, D..J.; Valero, V.; Durand, J.B.; Broglio, K.; Hess, K.R.; Michaud, L.B.; Gonzalez-Angulo, A.M.; Hortobagyi, G.N.; Esteva, F.J. Long-term cardiac tolerability of trastuzumab in metastatic breast cancer: The M.D. Anderson Cancer center experience. *J. Clin. Oncol.,* **2006**, *24*, 4107.

[123] Tan-Chiu, E.; Yothers, G.; Romond, E.; Geyer, C.E., Jr.; Ewer, M.; Keefe, D.; Shannon, R.P.; Swain, S.M.; Brown, A.; Fehrenbacher, L.; Vogel, V.G.; Seay, T.E.; Rastogi, P.; Mamounas, E.P.; Wolmark, N.; Bryant, J. Assessment of cardiac dysfunction in a randomized trial comparing doxorubicin and cyclophosphamide followed by paclitaxel, with or without trastuzumab as adjuvant therapy in node-positive, human epidermal growth factor receptor 2-overexpressing breast cancer: NSABP B-31. *J. Clin. Oncol.*, **2005**, *23*, 7811.

[124] Suter, T.M.; Procter, M.; van Veldhuisen, D.J. Trastuzumab associated cardiac adverse effects in the Herceptin adjuvant trial. *J. Clin. Oncol.,* **2007**, *25*, 3859.

[125] Hunt, S.A.; Baker, D.W.; Chin, M.H.; Cinquegrani, M.P.; Feldman, A.M.; Francis, G.S.; Ganiats, T.G.; Goldstein, S.; Gregoratos, G.; Jessup, M.L.; Noble, R.J.; Packer, M.; Silver, M.A.; Stevenson, L.W.; Gibbons, R.J.; Antman, E.M.; Alpert, J.S.; Faxon, D.P.; Fuster, V.; Gregoratos, G.; Jacobs, A.K.; Hiratzka, L.F.; Russell, R.O.; Smith, S.C., Jr. ACC/AHA guidelines for the evaluation and management of chronic heart failure in the adult: executive summary a report of the American College of Cardiology/ American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1995 Guidelines for the Evaluation and Management of Heart Failure): developed in collaboration with the International Society for Heart and Lung Transplantation, endorsed by the Heart Failure Society of America. *Circulation,* **2001**, *104*, 2996.

[126] Peng, X.; Chen, B.; Lim, C.C.; Sawyer. D.B. The Cardiotoxicology of anthracycline chemotherapeutics: Translating molecular mechanisms into preventative medicine. *Mol. Interv*., **2005***, 5*, 163.

[127] Gassmann, M.; Casagranda, F.; Orioli, D.; Simon, H.; Lai, C.; Klein, R.; Lemke, G. Aberrant neural and cardiac development in mice lacking the ERBB4 neuregulin receptor. *Nature,* **1995**, *378*, 390.

[128] Meyer, D.; Birchmeier, C. Multiple essential functions of neuregulin in development. *Nature,* **1995**, *378*, 386.
[129] Crone, S.A.; Zhao, Y.Y.; Fan, L.; Gu, Y.; Minamisawa, S.; Liu, Y.; Peterson, K.L.; Chen, J.; Kahn, R.; Condorelli, G.; Chien, K.R. Jr.; Lee, K.F. ERBB2 is essential in the prevention of dilated cardiomyopathy. *Nat. Med.,* **2002**, *8*, 459.
[130] Ozcelik, C.; Erdmann, B.; Pilz, B.; Wettschureck, N.; Britsch, S.; Hübner, N.; Chien, K.R.; Birchmeier, C.; Garratt, A.N. Conditional mutation of the ErbB2 (Her2) receptor in cardiomyocytes leads to dilated cardiomyopathy. *Proc. Natl. Acad. Sci. USA,* **2002**, *99*, 8880.
[131] Bersell, K.; Arab, S.; Haring, B.; Kuhn, B. Neuregulin1/ErbB4 signaling induces cardiomyocyte proliferation and repair of heart injury. *Cell,* **2009**, *138*, 257.
[132] Ewer, M.S.; Lippman, S.M. Type II chemotherapy-related cardiac dysfunction: time to recognize a new entity. *J. Clin. Oncol.,* **2005**, *23*, 2900.
[133] Chien, K.R. Herceptin and the heart: a molecular modifier of cardiac failure. *N. Engl. J. Med.,* **2006**, *354*, 789.
[134] Jones, L.W.; Haykowsky, M.J.; Swartz, J.J. Early breast cancer therapy and cardiovascular injury. *J. Am. Coll. Cardiol.,* **2007**, *50*, 1435.
[135] Force, T.; Krause, D.S.; Van Etten, R.A. Molecular mechanisms of cardiotoxicity of tyrosine kinase inhibition. *Nat. Rev. Cancer*, **2007**, *7*, 332.
[136] Motzer, R.J. Sunitinib versus interferon α in metastatic renal-cell carcinoma. *N. Engl. J. Med.,* **2007**, *356*,115.
[137] Demetri, G.D. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. *Lancet,* **2006**, *368*, 1329.
[138] Chu, T.F.; Rupnick, M.A.; Kerkela, R.; Dallabrida, S.M.; Zurakowski, D.; Nguyen, L.; Woulfe, K.; Pravda, E.; Cassiola, F.; Desai, J.; George, S.; Morgan, J.A.; Harris, D.M.; Ismail, N.S.; Chen, J.H.; Schoen, F.J.; Van den Abbeele, A.D.; Demetri, G.D.; Force, T.; Chen, M.H. Cardiotoxicity associated with tyrosine kinase inhibitor sunitinib. *Lancet*, **2007**, *370*, 2011.
[139] Pfizer. Sutent (sunitinib) PrescribingInformation Available from: <http://www.pfizer.com-pfizer/download/uspi_sutent.pdf>2007.
[140] Schmidinger, M.; Zielinski, C.C.; Vogl, U.M.; Schmidinger, H. Cardiac toxicity of sunitinib and sorafenib in patients with metastatic renal cell carcinoma. *J. Clin. Oncol.,* **2008**, *26*, 5204.
[141] Strevel, E.L.; Ing, D.J.; Siu, L.L. Molecularly Targeted Oncology Therapeutics and Prolongation of the QT Interval. *J. Clin. Oncol.*, **2007**, *25*, 3362.
[142] Mego, M.; Reckova, M.; Obertova, J.; Sycova-Mila, Z.; Brozmanova, K.; Mardiak, J. Increased cardiotoxicity of sorafenib in sunitinib-pretreated patients with metastatic renal cell carcinoma. *Ann. Oncol.*, **2007**, *18*, 1096.
[143] Buchdunger, E.; Zimmermann, J.; Mett, H.; Meyer, T.; Müller, M.; Druker, B.J.; Lydon, N. Inhibition of the Abl protein-tyrosine kinase *in vitro* and *in vivo* by a 2-phenylaminopyrimidine derivative. *Cancer Res*., **1996**, *56*, 100.
[144] Druker, B.J.; Tamura, S.; Buchdunger, E.; Ohno, S.; Segal, G.M.; Fanning, S.; Zimmerman, J.; Lydon, N.B. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat. Med*., **1996**, *2*, 561.
[145] Okuda, K.; Weisberg, E.; Gilliland, D.G.; Griffin, J.D. ARG tyrosine kinase activity is inhibited by STI571. *Blood*, **2001**, *97*, 2440.
[146] Kerkela, R.; Grazette, L.; Yacobi, R.; Iliescu, C.; Patten, R.; Beahm, C.; Walters, B.; Shevtsov, S.; Pesant, S.; Clubb, F.J.; Rosenzweig, A.; Salomon, R.N.; Van Etten, R.A.; Alroy, J.; Durand, J.B.; Force, T. Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. *Nat Med,* **2006**, *12*, 908.
[147] Ribeiro, A.L,; Marcolino, M.S.; Bittencourt, H.N.S.; Barbosa, M.M.; Nunes Mdo, C.; Xavier, V.F.; Clementino, N.C. An evaluation of the cardiotoxicity of imatinib mesylate. *Leuk. Res.*, **2008**, *32*, 1809.
[148] Force, T. In reply to "cardiotoxicity of the cancer therapeutic agent imatinib mesylate". *Nat. Med.*, **2007**, *13*, 15.
[149] Urano, F.; Wang, X.; Bertolotti, A.; Zhang, Y.; Chung, P.; Harding, H.P.; Ron, D. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science*, **2000**, *287*, 664.
[150] Tsuruta, F.; Sunayama, J.; Mori, Y.; Hattori, S.; Shimizu, S.; Tsujimoto, Y.; Yoshioka, K.; Masuyama, N.; Gotoh, Y. JNK promotes Bax translocation to mitochondria through phosphorylation of 14–3-3 proteins. *EMBO J.*, **2004**, *23*, 1889.
[151] Beltrami, A.P.; Barlucchi, L.; Torella, D.; Baker, M.; Limana, F.; Cimenti, S.; Kasahara, H.; Rota, M.; Musso, E.; Urbanek, K.; Leri, A.; Kajstura, J.; Nadal-Ginard, B.; Anversa, P. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell*, **2003**, *114*, 763.
[152] Chabot, B.; Stephenson, D.A.; Chapman, V.M.; Besmer, P.; Bernstein, A. The proto-oncogene c-kit encoding a transmembrane tyrosine kinase receptor maps to the mouse W locus. *Nature*, **1988**, *335*, 88.
[153] Bernex, F.; De Sepulveda, P.; Kress, C.; Elbaz, C.; Delouis, C. Spatial and temporal patterns of c-kit expressing cells in WlacZ/+ and WlacZ/ WlacZ mouse embryos. *Development,* **1996**, *122*, 3023.
[154] Waskow, C.; Paul, S.; Haller, C.; Gassmann, M.; Rodewald, H. Viable c-kit (W/W) mutants reveal pivotal role for c-kit in the maintenance of lymphopoiesis. *Immunity*, **2002**, *17,* 277.
[155] Cable, J.; Jackson, I.J.; Steel, K.P. Mutations at the W locus affects survival of neural crest-derived melanocytes in the mouse. *Mech. Dev.,* **1995**, *50*, 139.
[156] Schmelzle, T.; Hall, M.N. TOR, a central controller of cell growth. *Cell,* **2000**, *103*, 253.
[157] Blume-Jensen, P.; Jiang, G.; Hyman, R.; Lee, K.F.; O'Gorman, S.; Hunter, T. Kit/stem cell factor receptor-induced activation of phosphatidylinositol 3'-kinase is essential for male fertility. *Nat. Genet.,* **2000**, *24,* 157.
[158] Cramer, K.; Nieborowska-Skorska, M.; Koptyra, M.; Slupianek, A.; Penserga, E.T.; Eaves, CJ.; Aulitzky, W.; Skorski, T. BCR/ABL and other kinases from chronic myeloproliferative disorders stimulate single-strand annealing, and unfaithful DNA double strand break repair. *Cancer Res.,* **2008**, *68,* 6884.
[159] Suter, T.M.; Cook-Bruns, N.; Barton C. Cardiotoxicity associated with trastuzumab (Herceptin) therapy in the treatment of metastatic breast cancer. *Breast,* **2004**, *13*, 173.
[160] Chia, S.; Clemons, M.; Martin, L.A.; Rodgers, A.; Gelmon, K.; Pond, G.R.; Panasci, L. Pegylated liposomal doxorubicin and trastuzumab in HER-2 overexpressing metastatic breast cancer: a multicenter phase II trial. *J. Clin. Oncol.,* **2006**, *24*, 2773.
[161] Untch, M.; Eidtmann, H.; du Bois, A.; Meerpohl, H.G.; Thomssen, C.; Ebert, A.; Harbeck, N.; Jackisch, C.; Heilman, V.; Emons, G.; Wallwiener, D.; Wiese, W.; Blohmer, J.U.; Höffken, K.; Kuhn, W.; Reichardt, P.; Muscholl, M.; Pauschinger, M.; Langer, B.; Lück, H.J. Cardiac safety of trastuzumab in combination with epirubicin and cyclophosphamide in women with metastatic breast cancer: Results of a phase I trial. *Eur. J. Cancer,* **2004**, *40*, 988.
[162] Joensuu, H.; Kellokumpu-Lehtinen, P.L.; Bono, P.; Alanko, T.; Kataja, V.; Asola, R.; Utriainen, T.; Kokko, R.; Hemminki, A.; Tarkkanen, M.; Turpeenniemi-Hujanen, T.; Jyrkkiö, S.; Flander, M.; Helle, L.; Ingalsuo, S.; Johansson, K.; Jääskeläinen, AS.; Pajunen, M.; Rauhala, M.; Kaleva-Kerola, J.; Salminen, T.; Leinonen, M.; Elomaa, I.; Isola, J. FinHer Study Investigators. Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer. *N. Engl. J. Med.*, **2006**, *354*, 809.
[163] Van Royen, N.; Jaffe, C.C.; Krumholz, H.M.; Johnson, K.M.; Lynch, P.J.; Natale, D.; Atkinson, P.; Deman, P.; Wackers, F.J. Comparison and reproducibility of visual echocardiographic and quantitative radionuclide left ventricular ejection fractions. *Am. J. Cardiol.,* **1996**, *77*, 843.
[164] Bellenger, N.G.; Burgess, M.I.; Ray, S.G.; Lahiri, A.; Coats, A.J.; Cleland, J.G.; Pennell, D.J. Comparison of left ventricular ejection fraction and volumes in heart failure by echocardiography, radionuclide ventriculography and cardiovascular magnetic resonance: Are they interchangeable? *Eur. Heart J.*, **2000**, *21*, 1387.
[165] Godkar, D.; Bachu, K.; Dave, B.; Megna, R.; Niranjan, S.; Khanna, A. Comparison and co-relation of invasive and non-invasive methods of ejection fraction measurement. *J. Natl. Med. Assoc.,* **2007**, *99*, 1227.
[166] Mackey, J.R.; Clemons, M.; Côté, M.A.; Delgado, D.; Dent, S.; Paterson, A.; Provencher, L.; Sawyer, B.; Verma, S. Cardiac management during adjuvant trastuzumab therapy: recommendations of the Canadian Trastuzumab Working Group. *Curr. Oncol.,* **2008**, *15*, 24.
[167] Khakoo, A.Y.; Kassiotis, C.M.; Tannir, N.; Plana, J.C.; Halushka, M.; Bickford, C.; Trent, J. 2nd.; Champion, J.C.; Durand, J.B.;

Lenihan, D.J. Heart failure associated with sunitinib malate: a multitargeted receptor tyrosine kinase inhibitor. *Cancer*, **2008**, *112*, 2500.

[168] Joensuu, H. Cardiac toxicity of sunitinib. *Lancet*, **2007**, *370*, 1978.

[169] Ewer, M.S.; O'Shaughnessy, J.A. Cardiac toxicity of trastuzumab-related regimens in HER2-overexpressing breast cancer. *Clin. Breast Cancer,* **2007**, *7*, 600.

[170] Hunt, S.A.; Abraham, W.T.; Chin, M.H. ACC/AHA 2005 Guideline Update for the Diagnosis and Management of Chronic Heart Failure in the Adult: A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure) Developed in collaboration with the American College of Chest Physicians and the International Society for Heart and Lung Transplantation: Endorsed by the Heart Rhythm Society. *Circulation,* **2005**, *112*, e154.

[171] Izumiya, Y.; Shiojima, I.; Sato, K. Vascular endothelial growth factor blockade promotes the transition from compensatory cardiac hypertrophy to failure in response to pressure overload. *Hypertension*, **2006**, *47*, 887.

[172] Oliveira, P.J.; Goncalves, L.; Monteiro, P.; Providencia, L.A.; Moreno, A.J. Are the antioxidant properties of carvedilol important for the protection of cardiac mitochondria? *Curr. Vasc. Pharmacol.,* **2005**, *3*, 147.

[173] Carreira, R.S.; Monteiro, P.; Gon Alves, L.M.; Providência, L.A. Carvedilol: just another beta-blocker or a powerful cardioprotector? *Cardiovasc. Hematol. Disord. Drug Targets,* **2006**, *6,* 257.

[174] Jones, S.P.; Teshima, Y.; Akao, M. Simvastatin attenuates oxidant-induced mitochondrial dysfunction in cardiac myocytes. *Circ. Res*., **2003**, *93*, 697.

[175] Fernandez, A.; Sanguino, A.; Peng, Z.; Ozturk, E.; Chen, J.; Crespo, A.; Wulf, S.; Shavrin, A.; Qin, C.; Ma, J.; Trent, J.; Lin, Y.; Han, HD.; Mangala, LS. An anticancer C-Kit kinase inhibitor is reengineered to make it more active and less cardiotoxic. *J. Clin. Invest.,* **2007**, *117*, 3650.

Received: May 29, 2009 Revised: December 20, 2009 Accepted: December 22, 2009