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REVIEW

Recommendations for genetic testing to reduce the incidence of anthracycline-induced cardiotoxicity

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AIMS

Anthracycline-induced cardiotoxicity (ACT) occurs in 57% of treated patients and remains an important limitation of anthracycline-based chemotherapy. In various genetic association studies, potential genetic risk markers for ACT have been identified. Therefore, we developed evidence-based clinical practice recommendations for pharmacogenomic testing to further individualize therapy based on ACT risk.

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METHODS

We followed a standard guideline development process, including a systematic literature search, evidence synthesis and critical appraisal, and the development of clinical practice recommendations with an international expert group.

RESULTS

RARG rs2229774, *SLC28A3* rs7853758 and *UGT1A6* rs17863783 variants currently have the strongest and the most consistent evidence for association with ACT. Genetic variants in *ABCC1*, *ABCC2*, *ABCC5*, *ABCB1*, *ABCB4*, *CBR3*, *RAC2*, *NCF4*, *CYBA*, *GSTP1*, *CAT*, *SULT2B1*, *POR*, *HAS3*, *SLC22A7*, *SCL22A17*, *HFE* and *NOS3* have also been associated with ACT, but require additional validation. We recommend pharmacogenomic testing for the *RARG* rs2229774 (S427L), *SLC28A3* rs7853758 (L461L) and *UGT1A6*4* rs17863783 (V209V) variants in childhood cancer patients with an indication for doxorubicin or daunorubicin therapy (Level B – moderate). Based on an overall risk stratification, taking into account genetic and clinical risk factors, we recommend a number of management options including increased frequency of echocardiogram monitoring, follow-up, as well as therapeutic options within the current standard of clinical practice.

CONCLUSIONS

Existing evidence demonstrates that genetic factors have the potential to improve the discrimination between individuals at higher and lower risk of ACT. Genetic testing may therefore support both patient care decisions and evidence development for an improved prevention of ACT.

Introduction

Anthracyclines are highly effective anticancer drugs that have contributed to 5-year survival rates of over 80% for some cancer types [1, 2]. They are among the most commonly used agents for the treatment of adult and childhood leukaemia, lymphoma and various solid tumours including breast, ovarian and lung cancers as well as sarcomas. Anthracyclines block DNA and RNA synthesis by inhibiting the topoisomerase II enzyme. The ensuing disruption of DNA replication and transcription prevents the replication of rapidly dividing cells. Through the creation of iron-mediated free oxygen radicals, anthracyclines also damage DNA, proteins and cell membranes of rapidly dividing cells [3].

The clinical utility of anthracyclines is limited primarily by high inter-individual variability in cumulative dose-dependent cardiac toxicity known as anthracycline-induced cardiotoxicity (ACT). ACT is the deleterious effect of anthracyclines on normal cardiac function due to the toxic effect on cardiac muscles and their conducting system. The clinical diagnosis, classification and grading of ACT according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v3.0 is shown in Table 1 [4]. Although most studies use the definition of ACT based on CTCAE, the specific grading criteria varies between studies. ACT manifests as asymptomatic cardiac dysfunction in up to 57% of treated patients [5–8], and as restrictive or dilated cardiomyopathy resulting in congestive heart failure (CHF) in up to 16–20% of patients [9–12]. Anthracycline-induced CHF is often resistant to therapy and has a mortality rate of up to 79% [10, 13, 14]. While some patients tolerate high anthracycline doses without ACT, others are affected even at low doses. ACT can be divided into three types based on the temporal relationship to treatment: *acute/subacute cardiotoxicity* develops within a week of anthracycline administration, is rare (<1% of childhood cancer patients) and usually resolves after discontinuation of treatment [12, 13]; *early-onset chronic progressive cardiotoxicity* occurs within a year after completion of therapy and is observed in approximately 2% of treated children [15, 16]; and most commonly, ACT manifests as *late-onset chronic progressive cardiotoxicity* developing more than a year after therapy completion with up to 65% of patients affected [7, 8].

The pathophysiology of ACT is not fully understood. It is thought to be mediated in part by reactive oxygen species formed as a result of anthracycline treatment, leading to lipid peroxidation and DNA damage in cardiomyocytes [13, 17]. Other suggested causes of ACT include the accumulation of cardiotoxic anthracycline metabolites in the heart, disruption of calcium homeostasis, mitochondrial damage, and induction of apoptosis [13, 17–21]. Higher lifetime cumulative anthracycline dose, concurrent or prior cardiac irradiation, concomitant administration of other cardiotoxic chemotherapeutic agents (particularly paclitaxel, trastuzumab and cyclophosphamide), pre-existing cardiovascular disease, comorbidities (including renal dysfunction, pulmonary disease, infection, pregnancy), higher individual dose, shorter infusion time, age extremes (younger or elderly age at treatment), female sex, African American ancestry and Trisomy 21 [5, 9, 11, 13, 22–26] are known risk factors for ACT. The variable susceptibility to ACT, even when considering these clinical and demographic risk factors, suggests a genetic component. Candidate gene and genome-wide association studies have identified genetic variants associated with ACT [27–49]. However, no recommendations have been developed on the incorporation of genetic information into clinical therapeutic, management and follow-up decisions for cancer patients with an indication of anthracycline-based treatment regimens. Therefore, the intentions of this review were to: (1) review and summarize current evidence on genomic markers associated with ACT; (2) provide evidence-based recommendations as a basis for the use of a patient's genetic information to predict ACT risk and for guiding treatment, management and follow-up decisions; and (3) identify gaps

Table 1

Clinical characterization of anthracycline-induced cardiotoxicity

Patients	National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v3.0 [4].
No ACT	• **No cardiotoxicity**
	– SF ≥ 30%, ≥5yr follow-up
ACT	• **Grade 1 toxicity:**
	– Shortening fraction: 24% ≤ SF < 30%
	– Resting ejection fraction: 50% ≤ EF < 60%
	• **Grade 2 toxicity**: Moderate to severe cardiotoxicity
	– Shortening fraction: 15% ≤ SF < 24%
	– Resting ejection fraction: 40% ≤ EF < 50%
	• **Grade 3 toxicity**: Symptomatic congestive heart failure
	– Shortening fraction: SF < 15% or
	– Resting ejection fraction: 20% ≤ SF < 40%
	• **Grade 4 toxicity**: Congestive heart failure requiring heart transplant or ventricular assist device
	– Resting ejection fraction < 20%

in knowledge to prioritize future research. Specifically, recommendations for the following key questions were developed:

1. Should genetic testing be performed in patients with an indication for anthracycline therapy to predict risk of ACT? Which genetic test(s) should be performed and who should be tested?
2. How should patients with an indication for anthracycline therapy be managed based on the genetic test results?

These recommendations are intended to provide guidance on the use of pharmacogenomic testing to improve the management of ACT risk and reduce the occurrence of cardiotoxicity and congestive heart failure in patients receiving anthracyclines for their cancer treatment.

Methods

A standard guideline development process was followed, as previously described [50]. A comprehensive systematic search of the relevant English-language, published, peer-reviewed literature was performed to identify available evidence on the association of different genetic variants and ACT. Embase from the period 1974–June 2011 (using the OVID interface) and MEDLINE from the period 1946–July 2011 (using the OVID interface) were searched. Titles and abstracts of all records retrieved were scanned for relevance to the guideline key questions. English language original studies relevant to the guideline questions were selected for full-text review. Conference abstracts, editorials, notes, short surveys, and review articles were not included in the full-text review. All studies involving patients treated with anthracycline as part of their chemotherapy were included, whereas experimental *in vitro* and animal studies were excluded. The outcome of the studies included any clinical assessment of cardiac function (left ventricular (LV) ejection fraction (EF), ventricular shortening fraction (SF), LV volume, diastolic function, strain, molecular imaging, circulating biomarkers and others) and any grading of ACT (CTCAE and others). Study inclusion was not restricted with respect to the study design. Updates of the systematic literature search were performed until January 2016 (Supplementary Methods online). This was followed by an evaluation of the strength of evidence on pharmacogenomic markers for the prediction of ACT.

A level of evidence was assigned to each genetic biomarker, which reflects the consistency of independent study results, the magnitude of effect (e.g., reported as odds ratio), and the number and quality of studies conducted, expert clinical opinion and the deliberations of the CPNDS Clinical Recommendations Group (Table 2). The quality of individual studies and available evidence were assessed based on the quality of the clinical characterization (clinical and demographic information), the genotyping (e.g. call rates, reproducibility/replication error, Hardy-Weinberg equilibrium, cryptic relatedness verification and population stratification) and the data analysis and interpretation (statistical analytic approach and conclusions). Clinical practice recommendations were developed during a workshop meeting of recommendation development group members (Supplementary Methods online). Each clinical practice recommendation was assigned a level of strength, based on the strength of supporting evidence, the balance between benefits and risks of genotype-guided treatment, and the likelihood of variability in the individual values and preferences of patients (Table 3) [50]. Draft recommendations were submitted to a tiered review process, which included internal review by the Recommendation Group members, followed by external review by content experts and members of the intended target audience (Supplementary Methods online). Pharmacogenomic test performance measures (e.g. sensitivity, specificity, posttest probabilities) were

Table 2

Grading scheme used for critical appraisal of evidence

Grade	Results	Description
++++	Consistent, generalizable	Strong **general conclusions** can be drawn that are **unlikely to change** based on further research
+++	Consistent, but limited quantity, quality or generalizability	Evidence allows **general conclusions**, but with **reduced confidence**; further research is likely to have an important impact on confidence in conclusions
++	Inconsistent or insufficient quantity/quality, encouraging	No general conclusions can be drawn or conclusions are **likely to change** based on further research, but current evidence is **encouraging**
+	Inconsistent or insufficient quantity/quality, discouraging	No conclusions can be drawn or conclusions are **likely to change** based on future studies, and current evidence is **discouraging**

Table 3

Grading scheme used for grading of clinical practice recommendations

Level	Strength	Evidence basis
A	Strong	Based on **strong** scientific evidence; benefits clearly outweigh risks
B	Moderate	Based on **reduced confidence** scientific evidence and expert opinion; benefits likely to outweigh risks
C	Optional	Based mainly on **expert opinion**, for use with **evidence development** in a research context

calculated as described in the Supplementary Methods online for variants with a recommendation for testing.

The nomenclature of the drug and molecular targets including phase I and phase II drug metabolism enzymes, drug transporters, drug receptors, ion channels, transcription factors and other drug targets included in this review conforms to the *British Journal of Pharmacology*'s *Guide to Receptors and Channels* [51].

Results

Evidence synthesis and critical appraisal

Overall, existing evidence demonstrates that genetic factors have the potential to improve the discrimination between individuals at higher, moderate and lower risk for ACT. *RARG* rs2229774, *SLC28A3* rs7853758 and *UGT1A6*4* rs17863783 currently have the strongest evidence (+++ evidence) as pharmacogenomic markers for ACT [27–29]. Associations of these biomarkers with ACT have been consistently shown and replicated at least twice in large well-characterized patient populations with clinically relevant effect sizes (reported as odds ratios > 3 or < 0.3) [27–29]. Genetic variants in other genes (*ABCC1*, *ABCC2*, *ABCC5*, *ABCB1*, *ABCB4*, *CBR3*, *RAC2*, *NCF4*, *CYBA*, *GSTP1*, *CAT*, *SULT2B1*, *POR*, *HAS3*, *SLC22A7*, *SCL22A17*, *HFE* and *NOS3*) have also been associated with ACT, but these associations require additional validation (++/+ evidence) [30–49]. A brief summary of the evidence regarding these associations with ACT is provided below. A more detailed discussion of these potential genetic risk factors for ACT is provided online in the Supplementary Material and in the Supplementary Tables (S1-S14).

Retinoic acid receptor gamma (RARG). *RARG* has been shown to be involved in cardiac development and remodelling, which may implicate critical processes in the pathophysiology of ACT [52–58]. A recent genome-wide association study (GWAS) uncovered a non-synonymous coding variant (rs2229774, S427L) in *RARG* that was associated with ACT in children [29]. This association was replicated in European, African, East Asian, Hispanic and Aboriginal Canadian patient populations [29]. The *RARG* rs2229774 variant has been shown to alter RARG function leading to a reduced repression of the key ACT genetic determinant, *TOP2B* (Table S1) [29]. Although the number of studies is limited, the evidence for the role of *RARG* in ACT is consistent across different populations, and is further supported by mechanistic studies (+++ evidence).

Solute carrier (SLC) transporters. Two variants in the SLC transporter *SLC28A3* (rs7853758, rs885004) have shown consistent associations with doxorubicin and daunorubicin-induced cardiotoxicity in three independent well-characterized paediatric cohorts with the minor allele of the variant conferring a reduced ACT risk (+++ evidence; Table S2) [27, 28]. Current evidence indicates that the effect of *SLC28A3* may be specific to children receiving doxorubicin and daunorubicin, as two recent studies in adult cancer survivors did not detect any association with ACT [31, 32]. The two associated variants are in high linkage disequilibrium and rs7853758 has been associated with altered *SLC28A3* mRNA levels, suggesting a functional effect related to this synonymous (L461L) variant [59]. In addition, genetic associations of *SLC22A17* rs4982753 and *SLC22A7* rs4149178 have been discovered and replicated in large well-characterized paediatric patient populations (++ evidence) [30]. The known function of the SLC super family

as drug transporters (80) and the reported transport of anthracyclines by SLC transporters [60] provides biological support for these genetic associations.

UDP-glucuronosyltransferase family 1A, isoform 6 (UGT1A6). A synonymous coding variant (rs17863783, V209V) in *UGT1A6* showed evidence for an association with an increased risk of ACT in three independent paediatric patient populations (+++ evidence; Table S3) [27, 28]. Rs17863783 is a tag marker of the *UGT1A6*4* haplotype, which has been reported to cause a 30–100% reduction in enzyme activity [61, 62]. Given the role of *UGT1A6* in the drug detoxification glucuronidation pathway [63], reduced UGT1A6-mediated glucuronidation of anthracycline metabolites may lead to accumulation of toxic metabolites in patients carrying *UGT1A6*4*, resulting in the observed increased ACT risk.

ATP binding cassette (ABC) transporters. Associations of genetic polymorphisms in ABC transporter genes with ACT have been reported by several studies, including *ABCC1* (rs45511401, rs246221, rs4148350, rs246214), *ABCC2* (rs8187694-rs8187710 haplotype, rs4148391, rs4148399), *ABCC5* (rs7627754), *ABCB1* (rs2235047) and *ABCB4* (rs1149222, rs4148808) (Table S4) [27, 31, 33, 34, 36]. Only three of these associations (*ABCC1* rs246221, *ABCC2* rs8187694-rs8187710 haplotype and *ABCC5* rs7627754) have been replicated in independent cohorts [29, 31, 32, 35], but the consistency of findings, and the quantity and quality of the evidence remains limited. All other genetic associations in ABC transporters have not yet been replicated. Considering the role of ABC transporters in the transport of a variety of drugs including anthracyclines [64], more studies are required to clarify the relevance of these genetic variants in ACT (++ evidence).

Carbonyl reductases (CBR). The rs1056892 variant of *CBR3* was found to be associated with ACT in two paediatric studies [38, 39], with additional supporting evidence from a third investigation (Table S5) [40]. In addition, functional studies suggest an effect of this variant on the metabolism of doxorubicin into the cardiotoxic metabolite doxorubicinol by *CBR3* [39]. However, no association of rs1056892 with ACT was observed in other studies (Table S5) [27, 29, 35, 41]. Overall, the evidence regarding this association is thus inconsistent (++ evidence).

Nicotinamide adenine dinucleotide phosphate (NADPH) multienzymes complex. Evidence of genetic associations with polymorphisms in NAD(P)H oxidase subunits involved in the production of reactive oxygen species (ROS) have been reported. Associations for *NCF4* rs1883112, *CYBA* rs4673 and *RAC2* rs13058338 have been discovered and replicated at least once in independent studies (Table S6) [31, 33, 35, 37, 44]. In addition, it was shown that mice deficient in NAD(P)H oxidase activity were protected from the adverse cardiac effects of chronic doxorubicin treatment [33]. However, the reported genetic associations could not be replicated in other studies [27, 29, 31] and current evidence for genetic associations of NAD(P)H oxidase subunits with ACT thus remains limited and conflicting (++ evidence).

Glutathione S-transferase (GST) enzymes. An association of genetic variants in *GSTP1* with ACT has been reported in two small studies [40, 65], but has not been replicated elsewhere [27, 29, 37, 46], resulting in inconsistent evidence overall (++ evidence; Table S7).

Catalase (CAT) enzyme. A relatively small paediatric study (<100 patients) focused on genes involved in ROS metabolism identified an intronic variant (rs10836235) in *CAT* as marginally associated with ACT (Table S8) [47]. However, a recent GWAS in children did not find any associations with this and other variants in *CAT* with ACT [29]. Current evidence thus remains unclear (+ evidence).

Sulfotransferase family cytosolic 2B member 1 (SULT2B1) enzyme. An association of the rs10426377 variant in *SULT2B1* involved in the sulfate conjugation of drugs with ACT has been identified and replicated in independent paediatric patient populations [27, 28] (Table S9). The replication of *SULT2B1* rs10426377 was only marginally significant and additional evidence is required to confirm this association (++ evidence).

Hyaluronan synthase 3 (HAS3) enzyme. A coding variant in *HAS3* (rs2232228) was reported to be associated with cardiomyopathy with evidence of replication in a case-only cohort (Table S10) [36]. Specifically, an association of *HAS3* rs2232228 with the risk of ACT was observed in patients with high cumulative anthracycline exposure (>250 mg m^{-2}) [36]. Conversely, no association of *HAS3* variants was observed in a recent GWAS [29]. The association of *HAS3* rs2232228 thus requires further independent replication (++ evidence). However, the known role of HAS3 in cardiac remodelling [66] provides mechanistic and biological support for this genetic association.

Histamine N-methyltransferase (HNMT) enzyme. An association of *HNMT* rs17583889 with ACT has been reported in childhood cancer survivors [27–29]. In addition, the presence of the *HNMT* rs17583889 homozygous (high risk) and heterozygous (intermediate risk) genotypes, respectively, was detected in two adult sisters who developed cardiotoxicity after low dose doxorubicin treatment [48] (Table S11). However, the quantity of the evidence remains limited and additional studies are needed to further investigate the potential role of *HNMT* variants in ACT and to strengthen the confidence in this association (++ evidence).

Human haemochromatosis (HFE) protein. HFE deficiency increases the susceptibility to ACT [67]. The *HFE* variants rs1799945 (H63D) and rs1800562 (C282Y) have been associated with the risk of CHF and anthracycline-induced cardiac injury, respectively [35, 49] (Table S12). Furthermore, the association between anthracycline treatment and dose-dependent myocardial iron overload was shown to be modulated by *HFE* variants (C282Y and H63D) [43]. No additional studies have observed the association of *HFE* mutations with ACT to date (++ evidence).

Cytochrome P450 oxidoreductase (CYPOR/POR). Genetic associations with ACT for three intronic variants in *POR* (rs2868177, rs13240755, rs4732513) have been reported in a small study (<100 patients) of acute myeloid leukaemia patients receiving daunorubicin [42]. Conversely, a previous study investigating rs13240755 and rs4732513 did not find any association with ACT [27] (Table S13). The quantity and the strength of the evidence remain limited, thereby reducing the confidence in the associations (+ evidence). Considering the role of POR enzymes in the cytochrome P450 system and in the biotransformation of a variety of drugs including anthracyclines, more studies will be required to clarify the effect of these genetic variants in ACT.

Nitric oxide synthase 3 (NOS3) enzyme. A genetic association of *NOS3* rs1799983 with doxorubicin cardiotoxicity has been reported in a study that included survivors of childhood acute lymphoblastic leukaemia (Table S14) [68], but not in a recent GWAS that included other anthracycline and tumour types [29]. Despite the essential role of *NOS3* in cardiovascular function, the evidence for the association of *NOS3* polymorphism with ACT remains limited and inconsistent. Additional genetic and functional studies are needed to further clarify the relevance of the *NOS3* gene in ACT (+ evidence).

Clinical practice recommendations

The goals of these recommendations are to provide guidance on the use of pharmacogenomic testing to reduce the incidence of cardiotoxicity and congestive heart failure in patients that receive anthracycline chemotherapy for cancer treatment. Genetic information on risk of adverse effects are an important part of chemotherapy decision making; therefore these guidelines are designed to assist clinicians in the interpretation of genetic test results and in the use of this information in providing optimal clinical care for patients. They have been developed based on the quantity, quality and consistency of the current scientific evidence and the deliberations of the CPNDS clinical recommendations group.

Should genetic testing be performed in patients with an indication for anthracycline therapy to predict the risk of ACT? Which genetic test (s) should be performed and who should be tested?

Pharmacogenomic testing should be performed in all childhood cancer patients with an indication for doxorubicin or daunorubicin therapy for *RARG* rs2229774, *SLC28A3* rs7853758 and *UGT1A6*4* rs17863783 variants (Level B – moderate recommendation). Genetic testing is currently not recommended in adult patients and in children receiving other types of anthracyclines (Level C – optional recommendation).

Considerations: Current evidence regarding the association of these variants with ACT is consistent with a similar effect observed across studies conducted so far (+++ evidence). As the number of studies remains small (<5 studies), additional retrospective and prospective studies would further strengthen the confidence in the associations. Similarly, as all studies so far were performed in paediatric patients receiving primarily doxorubicin or daunorubicin, the generalizability of these findings to adult populations and other anthracyclines is unknown. Therefore, based on current evidence, this recommendation cannot be extrapolated to adults and to other anthracyclines.

How Should Patients be Managed Based on Genetic Testing Results?

Interpretation of the genetic test results. The *RARG* rs2229774 (G>A) risk variant (*A*-allele) and the *UGT1A6*4* rs17863783 (G>T) risk variant (*T*-allele) have been associated with significantly increased risk of developing ACT in childhood cancer survivors. Childhood cancer patients carrying the *RARG* rs2229774A or *UGT1A6*4* risk variants should therefore be considered at increased risk (high risk) of ACT compared to a classification based on clinical risk factors alone. The *SLC28A3* rs7853758 *A*-allele has been associated with a reduced risk of ACT. For patients carrying the rs7853758A protective variant who do not carry *RARG* rs2229774 or *UGT1A6*4* risk variants, classification into a lower ACT risk group should be considered. All other patients should be considered at moderate genetic risk. Predictive performance measures for *RARG* rs2229774, *SLC28A3* rs7853758 and *UGT1A6*4* rs17863783 are shown in Table 4 [29].

Management options based on ACT risk. The management of patients based on the recommendations below should be within the current standard of care guidelines, taking into consideration both the risk of cardiotoxicity and possible effects of management options on treatment effectiveness. Management options based on risk stratification also vary as evidence on specific treatment options may only be available for certain cancer types. The recommended management options address treatment considerations, monitoring and prevention and should be interpreted individually within the unique circumstances for each patient.

Low risk patients: normal follow-up (level A recommendation). Patients genetically and clinically determined to be at low risk of ACT should receive echocardiogram follow-up as usual. The Children's Oncology Group (COG) Long Term Follow-Up Guidelines v3.0 currently recommend cardiac follow-up every 5 years for those deemed to be low risk based on clinical factors [69, 70].

Moderate risk patients: increase frequency of monitoring (level A recommendation). Patients initially determined to be at low risk of ACT with a moderate genetic risk should receive increased echocardiogram follow-up and monitoring for cardiotoxicity. Based on COG guidelines for patients deemed to be at moderate ACT risk based on clinical risk factors [69, 70], we recommend cardiac follow-up every 2 years for patients with a moderate genetic risk.

High risk patients. For patients determined to be at high risk of ACT based on genetic testing and clinical risk factors, the following management options should be considered:

Table 4

Pharmacogenomic testing to assess the risk of ACT and inform treatment decisions in childhood cancer

Genetic marker	Sensitivity (95% CI)	Specificity (95% CI)	Positive post-test probability (%)	Negative post-test probability (%)	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR- (95% CI)	NNT
RARG rs2229774	45.7 (30.9–61)	86.3 (81.8–90)	55.1	18.9	34.4 (22.7–47.7)	90.9 (86.9–94.1)	3.3 (2.2–5.1)	0.63 (0.48–0.82)	3.6
UGT1A6*4 rs17863783	15.2 (6.3–28.9)	96.2 (93.3–98.1)	59.8	24.6	38.9 (17.3–64.3)	87.8 (83.7–91.2)	4.0 (1.6–9.9)	0.88 (0.78–1.00)	3.0
SLC28A3 rs7853758	17.4 (7.8–31.4)	64.6 (58.8–70.1)	15.4	32.1	7.2 (3.2–13.7)	83.2 (77.7–87.8)	0.49 (0.26–0.94)	1.30 (1.09–1.5)	8.6

Calculations were based on a well-characterized multiethnic population of 337 paediatric oncology patients treated with anthracycline chemotherapy [29], described in further detail in the Supplementary Methods online. Post-test probability is also known as exposure specific risk (absolute risk) and was estimated using the prevalence of ACT in childhood cancer survivors (27%) [6]. We used the 'number needed to treat (NNT)' to estimate the number of patients that need to be screened in order to prevent one case of ACT in childhood cancer. NNT = 1/ARR, where ARR is the absolute risk reduction. LR+ = positive likelihood ratio, LR− = negative likelihood ratio, NPV = negative predictive value, PPV = positive predictive value. Methodological details are available in the supplementary method online.

Increase frequency of monitoring (*level A recommendation*). Patients with a high risk of ACT should be followed more closely, with serial yearly echocardiographic monitoring and follow-up as recommended by COG guidelines [69, 70] for high risk anthracycline-treated childhood cancer survivors. High risk patients should also receive additional heart monitoring before each administration of anthracyclines.

Aggressive screening and management of cardiovascular risk factors (*level A recommendation*). In high risk patients, cardiovascular risk factors such as obesity, diabetes, arterial hypertension, coronary artery disease, lipid disorders and peripheral vascular disease should be screened regularly and treated aggressively.

Prescription of dexrazoxane (*level B recommendation*). Dexrazoxane is an iron chelator that protects the myocardium from oxidative damage. It is approved for cardioprotection in adult cancer patients but is also prescribed off-label to children. Several randomized control trials (RCTs) have shown that dexrazoxane is effective in preventing myocardial damage without compromising antitumour response and survival outcomes (Table S15) [71–82]. However, as a result of a suggested increased risk of secondary malignancies, the European Medicines Agency limited its indication to adult patients with advanced or metastatic breast cancer and contraindicated its use in children and adolescents [83]. Furthermore, the use of dexrazoxane can be associated with nausea, vomiting, stomatitis, diarrhoea, enhanced myelosuppression and other adverse effects. Therefore, we recommend its use when a high risk of ACT is expected with careful consideration of potential benefits and risks individually for each patient.

Use of liposomal encapsulated anthracycline preparations (*level C recommendation*). Liposomal formulations of daunorubicin and doxorubicin are thought to lower the amount of the drug that is delivered to the heart, potentially making treatment less cardiotoxic. Several clinical trials compared the efficacy, safety and antitumour response of liposomal anthracyclines to conventional preparations (Table S16). Liposomal doxorubicin was found to have similar efficacy and survival outcomes as regular doxorubicin but significantly lower risks of ACT and congestive heart failure in adult patients [84–87]. However, the number of studies is relatively small with a lack of long-term follow-up data, and no RCTs so far in children. Therefore, it is difficult to draw strong conclusions regarding the relative cardiac safety of these formulations. Nevertheless, available data indicates that liposomal anthracycline formulations may offer a clinical benefit for patients with a high ACT risk. We therefore recommend the use of liposomal anthracyclines in the context of well-designed clinical trials to further evaluate their benefits, safety and effects on antitumour response.

Use of continuous infusion or slower infusion rates (*level C recommendation*). Anthracycline administration by continuous intravenous infusion or with slower infusion rates have been used in an attempt to lower peak drug concentration and reduce ACT risk (Table S17). Three RCTs in adult cancer patients suggested that ACT can be reduced with the use of continuous intravenous infusion [88–90], whereas two RCTs in children did not find any cardioprotective advantage of this mode of administration [91, 92]. Similarly, studies with adult patients have suggested that prolonged administration

to minimize circulating dose volume may decrease the risk of ACT [23, 90, 91, 93–96]. However, no high quality evidence regarding the cardiac safety and effectiveness of slower infusion rates is available in children. Given the small number of studies conducted overall, no definitive conclusions can be made about the effect of anthracycline administration rate on ACT risk and drug effectiveness. We therefore recommend considering these alternative administration options only in the context of well-designed clinical trials to generate further evidence.

Use of less cardiotoxic types of anthracyclines (*level C recommendation*). Although there is some debate about the type of anthracycline used with significant differences between Europe and North America, the quantity and quality of evidence directly comparing different types of anthracyclines is very limited (Table S18). An RCT comparing chemotherapy response for epirubicin and doxorubicin treatment in advanced breast cancer patients found no difference in the efficacy, safety, response rates and survival outcomes between both treatments [97]. Another RCT comparing idarubicin and daunorubicin in the treatment of AML in childhood cancer patients reported a better efficacy for idarubicin but no difference in toxicity rates between treatment arms [98] (Table S18). Taken together, no definitive conclusions can be made about a possible reduction in ACT risk related to the use of different anthracycline types. Further evaluation in high-quality trials is needed to determine the potential of this management option for patients with high ACT risk.

Use of other cardioprotective agents (*level C recommendation*). Other cardioprotective drugs such as L-carnitine, probucol, deferoxamine, ethylenediaminetetraacetic acid (EDTA), coenzyme Q10, N-acetylcysteine, vitamin E, digoxin, enalapril, phenethylamines, superoxide dismutase, monohydroxyethylrutoside and other ACE inhibitors or beta-blockers have demonstrated significant cardioprotective effects [99–110] but have been less well investigated compared to dexrazoxane. RCTs have been performed for some of these agents [82, 103, 111–114]. Based on the quantity and quality of the evidence, no definitive conclusions can be made about their cardioprotective effects in ACT, effects on antitumour efficacy, adverse effects, and long-term cardiac safety. Therefore, we recommend these cardioprotective agents only for the purpose of generating further evidence.

Prescribe alternative chemotherapy regimens for certain tumour types where alternative regimens have been shown to be equally effective (*level C recommendation*). There is an ongoing COG trial evaluating the use of chemotherapy protocols without anthracyclines in children with acute lymphoblastic leukaemia (AALL0932). This trial and other similar trials are needed to ascertain whether alternative chemotherapy regimens have similar antitumour efficacy and survival outcomes compared to anthracycline-based chemotherapy. In the meantime, no conclusions can be drawn about whether this intervention can help prevent heart damage without reducing the antitumour efficacy of chemotherapy.

Discussion

Significant advances have been made with the discovery of pharmacogenomic biomarkers to predict ACT risk. However, inconsistent findings across studies and implementation in clinical practice remain a substantial challenge. In particular, the variability in the clinical diagnosis, classification and grading of ACT introduces heterogeneity between studies, which needs to be addressed. Differences in study design and data analysis approaches may also contribute to discrepant study results and make it difficult to compare and combine studies. The heterogeneity in findings between studies and the lack of independent replication may be related to the variability in study populations (e.g. adult vs. paediatric and different ethnic composition of cohorts), different types of cancer or anthracyclines studied and different chemotherapy protocols used in different studies. A number of other factors may be implicated as well, including the technology used for assessing cardiac toxicity, duration of follow-up and competing risk factors. Furthermore, many of the genetic association studies for ACT carried out so far were performed in relatively small cohorts with little or no independent replication and functional validation. It is thus essential to further validate these genetic findings in independent patient cohorts and assess their generalizability across different study populations and different types of cancers and anthracyclines and different treatment protocols. In addition, functional validation (e.g., *in vitro* functional studies and *in vivo* pharmacokinetic studies) could significantly strengthen the evidence for the role of specific genes or genetic variants in ACT as well as provide insight into potential novel preventive or therapeutic strategies for ACT.

Variable follow-up time between studies investigating ACT risk factors could also affect results and make study comparisons challenging as the relevance of specific genetic risk factors might differ between acute, early and late cardiotoxicity. ACT can occur at any time during or after treatment and defining a feasible time course for cardiac monitoring remains a key challenge. Currently, there is no consensus on the optimal monitoring regimen in patients receiving anthracycline therapy and studies evaluating monitoring regimens are lacking [115, 116]. Knowledge on the efficacy and cost-effectiveness of different monitoring strategies in the context of clinical and genetic risk factors would be beneficial to further tailor cardiac monitoring and long-term follow-up towards the individual needs of each patient.

Studies are needed to evaluate the association of *RARG* rs2229774, *SLC28A3* rs7853758 and *UGT1A6*4* with ACT in adult cancer patients. There is encouraging evidence about the potential role of several other genetic variants for ACT such as *ABCC1*, *ABCC2*, *ABCC5*, *ABCB1*, *ABCB4*, *CBR3*, *RAC2*, *NCF4*, *CYBA*, *GSTP1*, *CAT*, *SULT2B1*, *POR*, *HAS3*, *SLC22A7*, *SLC22A17*, *HFE* and *NOS3* [30–49]. Further studies should be conducted to strengthen the evidence, and assess their generalizability and clinical utility. Studies should also be performed that aim to identify additional, novel genetic factors for ACT to improve the sensitivity and specificity of current prediction models. So far, all of the association studies for ACT have been retrospective. To better assess the clinical utility of genetic tests, prospective studies are needed that investigate the genetic risk factors in the context of different management options. Similarly, evidence supporting management strategies to reduce ACT is limited and remains conflicting. Well-designed RCTs and other prospective studies

with long-term follow-up, and standardized monitoring and reporting of cardiac outcomes are needed to further evaluate preventive strategies. New techniques for assessing subtle changes in cardiac function may aid in assessing the impact of these interventions. Until such studies are completed, recommended management options are restricted to those available within current standards of care.

Conclusions

Anthracycline-induced cardiotoxicity is a common, complex and devastating adverse drug reaction (ADR), associated with substantial morbidity and mortality and increased health, psychological, social and economic burden for patients, their families and the health care system. The burgeoning evidence of the role of genetic factors is rapidly expanding our knowledge and ability to predict and manage ACT. However, the uptake of available genetic information in treatment and follow-up decisions is very limited. Also, no recommendations have been proposed for the incorporation of available genetic information into clinical therapeutic decisions. We performed a systematic review and developed a number of evidence-based recommendations to provide a useful reference tool to guide physicians, clinical pharmacologists and other healthcare professionals in translating the best available evidence into clinical practice. The hope is that these recommendations will provide the evidence needed to enable prioritized access to genetic testing for cancer patients prior to anthracycline-based chemotherapy.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: FA, CJD, APB, MRH and BCC have applied for patents based upon some of the work related to the predictive markers of ADRs to anthracyclines described in this review; SRR, SH, MJR, JWL, AS, SS, KAG, DB and UA declare no conflict of interest. The funding agencies had no role in study design; collection, analysis and interpretation of data; writing of the report; or the decision to submit the report for publication. The corresponding author had the final responsibility for the decision to submit for publication

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Contributors

The conception and design of the study was the work of FA, BCC, UA, SRR, CJDR and MRH. The systematic literature search was performed by FA, SH, and UA, FA, UA, SRR, SH, CJDR, DB, SS, KAG and BCC were responsible for the evidence synthesis and critical appraisal. FA, UA, SRR, APB, AS, CJDR, DB, SS, KAG, MRH, BCC and the CPNDS Clinical Recommendations Group developed the evidence-based clinical practice recommendations. The article was reviewed and revised by FA, SRR, MJR, SS, KAG, DB, UA, APB, AS, CJDR, MRH, BCC and the CPNDS Clinical Recommendations Group. All authors critically reviewed the report, made suggestions for improving the report for important intellectual content, and approved the final version of the manuscript.

References

1 Altekruse SF Kosary CL, Krapcho M, Neyman N, Aminou R, Waldron W, *et al*., eds. SEER Cancer Statistics Review 1975–2007. Bethesda: National Cancer Institute, 2010.

2 Ellison LF, Pogany L, Mery LS. Childhood and adolescent cancer survival: a period analysis of data from the Canadian Cancer Registry. Eur J Cancer 2007; 43: 1967–75.

3 Scully RE, Lipshultz SE. Anthracycline cardiotoxicity in long-term survivors of childhood cancer. Cardiovasc Toxicol 2007; 7: 122–8.

4 Cancer Therapy Evaluation Program – Common Terminology Criteria for Adverse Events – Version 3, 2003.

5 Kremer LC, van der Pal HJ, Offringa M, van Dalen EC, Voute PA. Frequency and risk factors of subclinical cardiotoxicity after anthracycline therapy in children: a systematic review. Ann Oncol 2002; 13: 819–29.

6 van der Pal HJ, van Dalen EC, Hauptmann M, Kok WE, Caron HN, van den Bos C, *et al*. Cardiac function in 5-year survivors of childhood cancer: a long-term follow-up study. Arch Intern Med 2010; 170: 1247–55.

7 Lipshultz SE, Colan SD, Gelber RD, Perez-Atayde AR, Sallan SE, Sanders SP. Late cardiac effects of doxorubicin therapy for acute lymphoblastic leukemia in childhood. N Engl J Med 1991; 324: 808–15.

8 Lipshultz SE, Lipsitz SR, Mone SM, Goorin AM, Sallan SE, Sanders SP, *et al*. Female sex and drug dose as risk factors for late cardiotoxic effects of doxorubicin therapy for childhood cancer. N Engl J Med 1995; 332: 1738–43.

9 Kremer LC, van Dalen EC, Offringa M, Voute PA. Frequency and risk factors of anthracycline-induced clinical heart failure in children: a systematic review. Ann Oncol 2002; 13: 503–12.

10 Lefrak EA, Pitha J, Rosenheim S, Gottlieb JA. A clinicopathologic analysis of adriamycin cardiotoxicity. Cancer 1973; 32: 302–14.

11 Von Hoff DD, Layard MW, Basa P, Davis HL Jr, Von Hoff AL, Rozencweig M, *et al*. Risk factors for doxorubicin-induced congestive heart failure. Ann Intern Med 1979; 91: 710–7.

12 Hershman DL, Shao T. Anthracycline cardiotoxicity after breast cancer treatment. Oncology (Williston Park) 2009; 23: 227–34.

13 Lipshultz SE, Alvarez JA, Scully RE. Anthracycline associated cardiotoxicity in survivors of childhood cancer. Heart 2008; 94: 525–33.

14 Felker GM, Thompson RE, Hare JM, Hruban RH, Clemetson DE, Howard DL, *et al*. Underlying causes and long-term survival in patients with initially unexplained cardiomyopathy. N Engl J Med 2000; 342: 1077–84.

15 Rodvold KA, Rushing DA, Tewksbury DA. Doxorubicin clearance in the obese. J Clin Oncol 1988; 6: 1321–7.

16 Kremer LC, van Dalen EC, Offringa M, Ottenkamp J, Voute PA. Anthracycline-induced clinical heart failure in a cohort of 607 children: long-term follow-up study. J Clin Oncol 2001; 19: 191–6.

17 Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev 2004; 56: 185–229.

18 Mordente A, Meucci E, Silvestrini A, Martorana GE, Giardina B. New developments in anthracycline-induced cardiotoxicity. Curr Med Chem 2009; 16: 1656–72.

19 Mushlin PS, Cusack BJ, Boucek RJ Jr, Andrejuk T, Li X, Olson RD. Time-related increases in cardiac concentrations of doxorubicinol could interact with doxorubicin to depress myocardial contractile function. Br J Pharmacol 1993; 110: 975–82.

20 Boucek RJ Jr, Olson RD, Brenner DE, Ogunbunmi EM, Inui M, Fleischer S. The major metabolite of doxorubicin is a potent inhibitor of membrane-associated ion pumps. A correlative study of cardiac muscle with isolated membrane fractions. J Biol Chem 1987; 262: 15851–6.

21 Olson RD, Mushlin PS, Brenner DE, *et al*. Doxorubicin cardiotoxicity may be caused by its metabolite, doxorubicinol. Proc Natl Acad Sci U S A 1988; 85: 3585–9.

22 Tukenova M, Guibout C, Oberlin O, Doyon F, Mousannif A, Haddy N, *et al*. Role of cancer treatment in long-term overall and cardiovascular mortality after childhood cancer. J Clin Oncol 2010; 28: 1308–15.

23 Barry E, Alvarez JA, Scully RE, Miller TL, Lipshultz SE. Anthracycline-induced cardiotoxicity: course, pathophysiology, prevention and management. Expert Opin Pharmacother 2007; 8: 1039–58.

24 Hasan S, Dinh K, Lombardo F, Kark J. Doxorubicin cardiotoxicity in African Americans. J Natl Med Assoc 2004; 96: 196–9.

25 Krischer JP, Epstein S, Cuthbertson DD, Goorin AM, Epstein ML, Lipshultz SE. Clinical cardiotoxicity following anthracycline treatment for childhood cancer: the Pediatric Oncology Group experience. J Clin Oncol 1997; 15: 1544–52.

26 Gottdiener JS, Appelbaum FR, Ferrans VJ, Deisseroth A, Ziegler J. Cardiotoxicity associated with high-dose cyclophosphamide therapy. Arch Intern Med 1981; 141: 758–63.

27 Visscher H, Ross CJ, Rassekh SR, Barhdadi A, Dube MP, Al-Saloos H, *et al*. Pharmacogenomic prediction of anthracycline-induced cardiotoxicity in children. J Clin Oncol 2012; 30: 1422–8.

28 Visscher H, Ross CJ, Rassekh SR, Sandor GS, Caron HN, van Dalen EC, *et al*. Validation of variants in SLC28A3 and UGT1A6 as genetic markers predictive of anthracycline-induced cardiotoxicity in children. Pediatr Blood Cancer 2013; 60: 1375–81.

29 Aminkeng F, Bhavsar AP, Visscher H, Rassekh SR, Li Y, Lee JW, *et al*. A coding variant in RARG confers susceptibility to anthracycline-induced cardiotoxicity in childhood cancer. Nat Genet 2015; 47: 1079–84.

30 Visscher H, Rassekh SR, Sandor GS, Caron HN, van Dalen EC, Kremer LC, *et al*. Genetic variants in SLC22A17 and SLC22A7 are associated with anthracycline-induced cardiotoxicity in children. Pharmacogenomics 2015; 16: 1065–76.

31 Vulsteke C, Pfeil AM, Maggen C, Schwenkglenks M, Pettengell R, Szucs TD, *et al*. Clinical and genetic risk factors for epirubicin-induced cardiac toxicity in early breast cancer patients. Breast Cancer Res Treat 2015; 152: 67–76.

32 Reichwagen A, Ziepert M, Kreuz M, Godtel-Armbrust U, Rixecker T, Poeschel V, *et al*. Association of NADPH oxidase polymorphisms with anthracycline-induced cardiotoxicity in the RICOVER-60 trial of patients with aggressive CD20(+) B-cell lymphoma. Pharmacogenomics 2015; 16: 361–72.

33 Wojnowski L, Kulle B, Schirmer M, Schluter G, Schmidt A, Rosenberger A, *et al*. NAD(P)H oxidase and multidrug resistance protein genetic polymorphisms are associated with doxorubicin-induced cardiotoxicity. Circulation 2005; 112: 3754–62.

34 Semsei AF, Erdelyi DJ, Ungvari I, Csagoly E, Hegyi MZ, Kiszel PS, *et al*. ABCC1 polymorphisms in anthracycline-induced cardiotoxicity in childhood acute lymphoblastic leukaemia. Cell Biol Int 2012; 36: 79–86.

35 Armenian SH, Ding Y, Mills G, Sun C, Venkataraman K, Wong FL, *et al*. Genetic susceptibility to anthracycline-related congestive heart failure in survivors of haematopoietic cell transplantation. Br J Haematol 2013; 163: 205–13.

36 Wang X, Liu W, Sun CL, Armenian SH, Hakonarson H, Hageman L, *et al*. Hyaluronan synthase 3 variant and anthracycline-related cardiomyopathy: a report from the Children's Oncology Group. J Clin Oncol 2014; 32: 647–53.

37 Rossi D, Rasi S, Franceschetti S, Capello D, Castelli A, De Paoli L, *et al*. Analysis of the host pharmacogenetic background for prediction of outcome and toxicity in diffuse large B-cell lymphoma treated with R-CHOP21. Leukemia 2009; 23: 1118–26.

38 Blanco JG, Leisenring WM, Gonzalez-Covarrubias VM, Kawashima TI, Davies SM, Relling MV, *et al*. Genetic polymorphisms in the carbonyl reductase 3 gene CBR3 and the NAD(P)H:quinone oxidoreductase 1 gene NQO1 in patients who developed anthracycline-related congestive heart failure after childhood cancer. Cancer 2008; 112: 2789–95.

39 Blanco JG, Sun CL, Landier W, Chen L, Esparza-Duran D, Leisenring W, *et al*. Anthracycline-related cardiomyopathy after childhood cancer: role of polymorphisms in carbonyl reductase genes – a report from the Children's Oncology Group. J Clin Oncol 2012; 30: 1415–21.

40 Volkan-Salanci B, Aksoy H, Kiratli PO, Tulumen E, Guler N, Oksuzoglu B, *et al*. The relationship between changes in functional cardiac parameters following anthracycline therapy and carbonyl reductase 3 and glutathione S transferase Pi polymorphisms. J Chemother 2012; 24: 285–91.

41 Lubieniecka JM, Liu J, Heffner D, Graham J, Reid R, Hogge D, *et al*. Single-nucleotide polymorphisms in aldo-keto and carbonyl reductase genes are not associated with acute cardiotoxicity after daunorubicin chemotherapy. Cancer Epidemiol Biomarkers Prev 2012; 21: 2118–20.

42 Lubieniecka JM, Graham J, Heffner D, Mottus R, Reid R, Hogge D, *et al*. A discovery study of daunorubicin induced

cardiotoxicity in a sample of acute myeloid leukemia patients prioritizes P450 oxidoreductase polymorphisms as a potential risk factor. Front Genet 2013; 4: 231.

43 Cascales A, Sanchez-Vega B, Navarro N, Pastor-Quirante F, Corral J, Vicente V, *et al*. Clinical and genetic determinants of anthracycline-induced cardiac iron accumulation. Int J Cardiol 2012; 154: 282–6.

44 Cascales A, Pastor-Quirante F, Sanchez-Vega B, Luengo-Gil G, Corral J, Ortuno-Pacheco G, *et al*. Association of anthracycline-related cardiac histological lesions with NADPH oxidase functional polymorphisms. Oncologist 2013; 18: 446–53.

45 Windsor RE, Strauss SJ, Kallis C, Wood NE, Whelan JS. Germline genetic polymorphisms may influence chemotherapy response and disease outcome in osteosarcoma: a pilot study. Cancer 2012; 118: 1856–67.

46 Vivenza D, Feola M, Garrone O, Monteverde M, Merlano M, Lo NC. Role of the renin-angiotensin-aldosterone system and the glutathione S-transferase Mu, Pi and Theta gene polymorphisms in cardiotoxicity after anthracycline chemotherapy for breast carcinoma. Int J Biol Markers 2013; 28: e336–47.

47 Rajic V, Aplenc R, Debeljak M, Prestor VV, Karas-Kuzelicki N, Mlinaric-Rascan I, *et al*. Influence of the polymorphism in candidate genes on late cardiac damage in patients treated due to acute leukemia in childhood. Leuk Lymphoma 2009; 50: 1693–8.

48 Sachidanandam K, Gayle AA, Robins HI, Kolesar JM. Unexpected doxorubicin-mediated cardiotoxicity in sisters: possible role of polymorphisms in histamine n-methyl transferase. J Oncol Pharm Pract 2013; 19: 269–72.

49 Lipshultz SE, Lipsitz SR, Kutok JL, Miller TL, Colan SD, Neuberg DS, *et al*. Impact of hemochromatosis gene mutations on cardiac status in doxorubicin-treated survivors of childhood high-risk leukemia. Cancer 2013; 119: 3555–62.

50 Amstutz U, Shear NH, Rieder MJ, Hwang S, Fung V, Nakamura H, *et al*. Recommendations for HLA-B*15:02 and HLA-A*31:01 genetic testing to reduce the risk of carbamazepine-induced hypersensitivity reactions. Epilepsia 2014; 55: 496–506.

51 Alexander SP, Mathie A, Peters JA. Guide to Receptors and Channels (GRAC), 5th edition. Br J Pharmacol 2011; 164 (Suppl 1): S1–S324.

52 Kashyap V, Laursen KB, Brenet F, Viale AJ, Scandura JM, Gudas LJ. RARgamma is essential for retinoic acid induced chromatin remodeling and transcriptional activation in embryonic stem cells. J Cell Sci 2013; 126: 999–1008.

53 Purton LE, Dworkin S, Olsen GH, *et al*. RARgamma is critical for maintaining a balance between hematopoietic stem cell self-renewal and differentiation. J Exp Med 2006; 203: 1283–93.

54 Duester G. Retinoid signaling in control of progenitor cell differentiation during mouse development. Semin Cell Dev Biol 2013; 24: 694–700.

55 Iulianella A, Lohnes D. Chimeric analysis of retinoic acid receptor function during cardiac looping. Dev Biol 2002; 247: 62–75.

56 Niederreither K, Vermot J, Messaddeq N, Schuhbaur B, Chambon P, Dolle P. Embryonic retinoic acid synthesis is essential for heart morphogenesis in the mouse. Development 2001; 128: 1019–31.

57 Kikuchi K, Holdway JE, Major RJ, Blum N, Dahn RD, Begemann G, *et al*. Retinoic acid production by endocardium and epicardium is an injury response essential for zebrafish heart regeneration. Dev Cell 2011; 20: 397–404.

58 Bilbija D, Haugen F, Sagave J, Baysa A, Bastani N, Levy FO, *et al*. Retinoic acid signalling is activated in the postischemic heart and may influence remodelling. PLoS One 2012; 7: e44740.

59 Zeller T, Wild P, Szymczak S, Rotival M, Schillert A, Castagne R, *et al*. Genetics and beyond – the transcriptome of human monocytes and disease susceptibility. PLoS One 2010; 5: e10693.

60 Nagasawa K, Nagai K, Ohnishi N, Yokoyama T, Fujimoto S. Contribution of specific transport systems to anthracycline transport in tumor and normal cells. Curr Drug Metab 2001; 2: 355–66.

61 Nagar S, Zalatoris JJ, Blanchard RL. Human UGT1A6 pharmacogenetics: identification of a novel SNP, characterization of allele frequencies and functional analysis of recombinant allozymes in human liver tissue and in cultured cells. Pharmacogenetics 2004; 14: 487–99.

62 Krishnaswamy S, Hao Q, Al-Rohaimi A, Hesse LM, von Moltke LL, Greenblatt DJ, *et al*. UDP glucuronosyltransferase (UGT) 1A6 pharmacogenetics: II. Functional impact of the three most common nonsynonymous UGT1A6 polymorphisms (S7A, T181A, and R184S). J Pharmacol Exp Ther 2005; 313: 1340–6.

63 Bock KW, Kohle C. UDP-glucuronosyltransferase 1A6: structural, functional, and regulatory aspects. Methods Enzymol 2005; 400: 57–75.

64 Smith AJ, van Helvoort A, van Meer G, Szabo K, Welker E, Szakacs G, *et al*. MDR3 P-glycoprotein, a phosphatidylcholine translocase, transports several cytotoxic drugs and directly interacts with drugs as judged by interference with nucleotide trapping. J Biol Chem 2000; 275: 23530–9.

65 Windsor RE, Strauss SJ, Kallis C, Wood NE, Whelan JS. Germline genetic polymorphisms may influence chemotherapy response and disease outcome in osteosarcoma: a pilot study. Cancer 2011; 118: 1856–67.

66 Corda S, Samuel JL, Rappaport L. Extracellular matrix and growth factors during heart growth. Heart Fail Rev 2000; 5: 119–30.

67 Miranda CJ, Makui H, Soares RJ, Bilodeau M, Mui J, Vali H, *et al*. Hfe deficiency increases susceptibility to cardiotoxicity and exacerbates changes in iron metabolism induced by doxorubicin. Blood 2003; 102: 2574–80.

68 Krajinovic M, Elbared J, Drouin S, Bertout L, Rezgui A, Ansari M, *et al*. Polymorphisms of ABCC5 and NOS3 genes influence doxorubicin cardiotoxicity in survivors of childhood acute lymphoblastic leukemia. Pharmacogenomics J 2015. doi:10.1038/tpj.2015.63.

69 Children's Oncology Group. Long-term follow-up guidelines for survivors of childhood, adolescent and young adult cancers, Version 3.0. Arcadia, CA: Children's Oncology Group. Available at http://www.survivorshipguidelines.org (accessed October 2008).

70 Ryerson AB, Border WL, Wasilewski-Masker K, Goodman M, Meacham L, Austin H, *et al*. Assessing anthracycline-treated childhood cancer survivors with advanced stress echocardiography. Pediatr Blood Cancer 2015; 62: 502–8.

71 Wang P, Zhang S, Zhang XB, Li WJ, Hao XM, Zhang J. Protective effect of dexrazoxane on cardiotoxicity in breast cancer patients who received anthracycline-containing chemotherapy. Zhonghua zhong liu za zhi [Chinese Journal of Oncology]2013; 35: 135–9.

72 Lipshultz SE, Rifai N, Dalton VM, Levy DE, Silverman LB, Lipsitz SR, *et al*. The effect of dexrazoxane on myocardial injury in

doxorubicin-treated children with acute lymphoblastic leukemia. N Engl J Med 2004; 351: 145–53.

73 Lipshultz SE, Scully RE, Lipsitz SR, Sallan SE, Silverman LB, Miller TL, *et al.* Assessment of dexrazoxane as a cardioprotectant in doxorubicin-treated children with high-risk acute lymphoblastic leukaemia: long-term follow-up of a prospective, randomised, multicentre trial. Lancet Oncol 2010; 11: 950–61.

74 Wexler LH, Andrich MP, Venzon D, Berg SL, Weaver-McClure L, Chen CC, *et al.* Randomized trial of the cardioprotective agent ICRF-187 in pediatric sarcoma patients treated with doxorubicin. J Clin Oncol 1996; 14: 362–72.

75 Galetta F, Franzoni F, Cervetti G, Cecconi N, Carpi A, Petrini M, *et al.* Effect of epirubicin-based chemotherapy and dexrazoxane supplementation on QT dispersion in non-Hodgkin lymphoma patients. Biomed Pharmacother 2005; 59: 541–4.

76 Lopez M, Vici P, Di Lauro K, Conti F, Paoletti G, Ferraironi A, *et al.* Randomized prospective clinical trial of high-dose epirubicin and dexrazoxane in patients with advanced breast cancer and soft tissue sarcomas. J Clin Oncol 1998; 16: 86–92.

77 Marty M, Espie M, Llombart A, Monnier A, Rapoport BL, Stahalova V. Multicenter randomized phase III study of the cardioprotective effect of dexrazoxane (Cardioxane) in advanced/metastatic breast cancer patients treated with anthracycline-based chemotherapy. Ann Oncol 2006; 17: 614–22.

78 Swain SM, Whaley FS, Gerber MC, Weisberg S, York M, Spicer D, *et al.* Cardioprotection with dexrazoxane for doxorubicin-containing therapy in advanced breast cancer. J Clin Oncol 1997; 15: 1318–32.

79 Speyer JL, Green MD, Zeleniuch-Jacquotte A, Wernz JC, Rey M, Sanger J, *et al.* ICRF-187 permits longer treatment with doxorubicin in women with breast cancer. J Clin Oncol 1992; 10: 117–27.

80 Venturini M, Michelotti A, Del Mastro L, Gallo L, Carnino F, Garrone O, *et al.* Multicenter randomized controlled clinical trial to evaluate cardioprotection of dexrazoxane versus no cardioprotection in women receiving epirubicin chemotherapy for advanced breast cancer. J Clin Oncol 1996; 14: 3112–20.

81 Barry EV, Vrooman LM, Dahlberg SE, Neuberg DS, Asselin BL, Athale UH, *et al.* Absence of secondary malignant neoplasms in children with high-risk acute lymphoblastic leukemia treated with dexrazoxane. J Clin Oncol 2008; 26: 1106–11.

82 van Dalen EC, Caron HN, Dickinson HO, Kremer LC. Cardioprotective interventions for cancer patients receiving anthracyclines. Cochrane Database Syst Rev 2011; CD003917.

83 European Medicines Agency recommends restricting the use of dexrazoxane-containing medicines. Press release, 23 June 2011; EMA/491205/2011; Press Office.

84 Batist G, Ramakrishnan G, Rao CS, Chandrasekharan A, Gutheil J, Guthrie T, *et al.* Reduced cardiotoxicity and preserved antitumor efficacy of liposome-encapsulated doxorubicin and cyclophosphamide compared with conventional doxorubicin and cyclophosphamide in a randomized, multicenter trial of metastatic breast cancer. J Clin Oncol 2001; 19: 1444–54.

85 Harris L, Batist G, Belt R, Rovira D, Navari R, Azarnia N, *et al.* Liposome-encapsulated doxorubicin compared with conventional doxorubicin in a randomized multicenter trial as first-line therapy of metastatic breast carcinoma. Cancer 2002; 94: 25–36.

86 O'Brien ME, Wigler N, Inbar M, Rosso R, Grischke E, Santoro A, *et al.* 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: 440–9.

87 Safra T. Cardiac safety of liposomal anthracyclines. Oncologist 2003; 8 (Suppl 2): 17–24.

88 Hortobagyi GN, Frye D, Buzdar AU, Ewer MS, Fraschini G, Hug V, *et al.* Decreased cardiac toxicity of doxorubicin administered by continuous intravenous infusion in combination chemotherapy for metastatic breast carcinoma. Cancer 1989; 63: 37–45.

89 Shapira J, Gotfried M, Lishner M, Ravid M. Reduced cardiotoxicity of doxorubicin by a 6-hour infusion regimen. A prospective randomized evaluation. Cancer 1990; 65: 870–3.

90 Legha SS, Benjamin RS, Mackay B, Yap HY, Wallace S, Ewer M, *et al.* Adriamycin therapy by continuous intravenous infusion in patients with metastatic breast cancer. Cancer 1982; 49: 1762–6.

91 Lipshultz SE, Giantris AL, Lipsitz SR, Kimball Dalton V, Asselin BL, Barr RD, *et al.* Doxorubicin administration by continuous infusion is not cardioprotective: the Dana-Farber 91-01 Acute Lymphoblastic Leukemia protocol. J Clin Oncol 2002; 20: 1677–82.

92 Levitt GA, Dorup I, Sorensen K, Sullivan I. Does anthracycline administration by infusion in children affect late cardiotoxicity? Br J Haematol 2004; 124: 463–8.

93 Dillenburg RF, Nathan P, Mertens L. Educational paper: Decreasing the burden of cardiovascular disease in childhood cancer survivors: an update for the pediatrician. Eur J Pediatr 2013; 172: 1149–60.

94 Forssen EA, Tokes ZA. *In vitro* and *in vivo* studies with adriamycin liposomes. Biochem Biophys Res Commun 1979; 91: 1295–301.

95 Wouters KA, Kremer LC, Miller TL, Herman EH, Lipshultz SE. Protecting against anthracycline-induced myocardial damage: a review of the most promising strategies. Br J Haematol 2005; 131: 561–78.

96 van Dalen EC, Michiels EM, Caron HN, Kremer LC. Different anthracycline derivatives for reducing cardiotoxicity in cancer patients. Cochrane Database Syst Rev 2006; CD005006.

97 Perez DJ, Harvey VJ, Robinson BA, Atkinson CH, Dady PJ, Kirk AR, *et al.* A randomized comparison of single-agent doxorubicin and epirubicin as first-line cytotoxic therapy in advanced breast cancer. J Clin Oncol 1991; 9: 2148–52.

98 Creutzig U, Ritter J, Zimmermann M, Hermann J, Gadner H, Sawatzki DB, *et al.* Idarubicin improves blast cell clearance during induction therapy in children with AML: results of study AML-BFM 93. AML-BFM Study Group. Leukemia 2001; 15: 348–54.

99 De Leonardis V, Neri B, Bacalli S, Cinelli P. Reduction of cardiac toxicity of anthracyclines by L-carnitine: preliminary overview of clinical data. Int J Clin Pharmacol Res 1985; 5: 137–42.

100 Elihu N, Anandasbapathy S, Frishman WH. Chelation therapy in cardiovascular disease: ethylenediaminetetraacetic acid, deferoxamine, and dexrazoxane. J Clin Pharmacol 1998; 38: 101–5.

101 Garbrecht M, Mullerleile U. Verapamil in the prevention of adriamycin-induced cardiomyopathy. Klin Wochenschr 1986; 64 (Suppl 7): 132–4.

102 Guthrie D, Gibson AL. Doxorubicin cardiotoxicity: possible role of digoxin in its prevention. Br Med J 1977; 2: 1447–9.

103 Iarussi D, Auricchio U, Agretto A, Murano A, Giuliano M, Casale F, *et al*. Protective effect of coenzyme Q10 on anthracyclines cardiotoxicity: control study in children with acute lymphoblastic leukemia and non-Hodgkin lymphoma. Mol Aspects Med 1994; 15 (Suppl): s207–12.

104 Kawasaki S, Akiyama S, Kurokawa T, Kataoka M, Dohmitsu K, Kondoh K, *et al*. Polyoxyethylene-modified superoxide dismutase reduces side effects of adriamycin and mitomycin C. Jpn J Cancer Res: Gann 1992; 83: 899–906.

105 Legha SS, Benjamin RS, Mackay B, Ewer M, Wallace S, Valdivieso M, *et al*. Reduction of doxorubicin cardiotoxicity by prolonged continuous intravenous infusion. Ann Intern Med 1982; 96: 133–9.

106 Silber JH, Cnaan A, Clark BJ, Paridon SM, Chin AJ, Rychik J, *et al*. Design and baseline characteristics for the ACE Inhibitor After Anthracycline (AAA) study of cardiac dysfunction in long-term pediatric cancer survivors. Am Heart J 2001; 142: 577–85.

107 Silber JH, Cnaan A, Clark BJ, Paridon SM, Chin AJ, Rychik J, *et al*. Enalapril to prevent cardiac function decline in long-term survivors of pediatric cancer exposed to anthracyclines. J Clin Oncol 2004; 22: 820–8.

108 Singal PK, Siveski-Iliskovic N, Hill M, Thomas TP, Li T. Combination therapy with probucol prevents adriamycin-induced cardiomyopathy. J Mol Cell Cardiol 1995; 27: 1055–63.

109 Unverferth DV, Fertel RH, Balcerzak SP, Magorien RD, O'Dorisio MS. N-acetylcysteine prevents the doxorubicin-induced decrease of cyclic GMP. Semin Oncol 1983; 10: 49–52.

110 van Acker FA, van Acker SA, Kramer K, Haenen GR, Bast A, van der Vijgh WJ. 7-monohydroxyethylrutoside protects against chronic doxorubicin-induced cardiotoxicity when administered only once per week. Clin Cancer Res 2000; 6: 1337–41.

111 Kalay N, Basar E, Ozdogru I, Er O, Cetinkaya Y, Dogan A, *et al*. Protective effects of carvedilol against anthracycline-induced cardiomyopathy. J Am Coll Cardiol 2006; 48: 2258–62.

112 Kraft J, Grille W, Appelt M, Hossfeld DK, Eichelbaum M, Koslowski B, *et al*. Effects of verapamil on anthracycline-induced cardiomyopathy: preliminary results of a prospective multicenter trial. Haematol Blood Transfus 1990; 33: 566–70.

113 Milei J, Marantz A, Ale J, Vazquez A, Buceta JE. Prevention of adriamycin-induced cardiotoxicity by prenylamine: a pilot double blind study. Cancer Drug Deliv 1987; 4: 129–36.

114 Wagdi P, Rouvinez G, Fluri M, Aeschbacher B, Thoni A, Schefer H, *et al*. Cardioprotection in chemo- and radiotherapy for malignant diseases – an echocardiographic pilot study. Praxis 1995; 84: 1220–3.

115 Jannazzo A, Hoffman J, Lutz M. Monitoring of anthracycline-induced cardiotoxicity. Ann Pharmacother 2008; 42: 99–104.

116 Adams MJ, Lipshultz SE. Pathophysiology of anthracycline- and radiation-associated cardiomyopathies: implications for screening and prevention. Pediatr Blood Cancer 2005; 44: 600–6.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

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