Systems biology approaches to adverse drug effects: the example of cardio-oncology

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Abstract | Increased awareness of the cardiovascular toxic effects of chemotherapy has led to the emergence of cardio-oncology (or onco-cardiology), which focuses on screening, monitoring and treatment of patients with cardiovascular dysfunctions resulting from chemotherapy. Anthracyclines, such as doxorubicin, and HER2 inhibitors, such as trastuzumab, both have cardiotoxic effects. The biological rationale, mechanisms of action and cardiotoxicity profiles of these two classes of drugs, however, are completely different, suggesting that cardiotoxic effects can occur in a range of different ways. Advances in genomics and proteomics have implicated several genomic variants and biological pathways that can influence the susceptibility to cardiotoxicity from these, and other drugs. Established pathways include multidrug resistance proteins, energy utilization pathways, oxidative stress, cytoskeletal regulation and apoptosis. Gene-expression profiles that have revealed perturbed pathways have vastly increased our knowledge of the complex processes involved in crosstalk between tumours and cardiac function. Utilization of mathematical and computational modelling can complement pharmacogenomics and improve individual patient outcomes. Such endeavours should enable identification of variations in cardiotoxicity, particularly in those patients who are at risk of not recovering, even with the institution of cardioprotective therapy. The application of systems biology holds substantial potential to advance our understanding of chemotherapy-induced cardiotoxicity.

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Introduction

More than 40,000 deaths per year result from any adverse drug reaction in the USA alone, including from drug-induced cardiovascular toxicity.[1] Cardiotoxicity often occurs as a complication of chemotherapy and most commonly manifests as congestive heart failure, but can also present as ischaemia in multiple vascular territories, venous thromboembolism, hypertension, QT interval prolongation or arrhythmias.[2–4]

Herein we review the utility of systems-based approaches such as genomic profiling, candidate gene studies, genome-wide association studies (GWAS), proteomics and mathematical and computational modelling, and describe the insights these techniques provide into mechanisms of chemotherapy-induced cardiotoxicity as well as the foundation for developing novel cardioprotection strategies. Use of systems-based approaches might also enable identification of patients who are at the highest risk of chemotherapy-induced cardiotoxicity, and therefore lead to more-effective resource allocation for patient screening and intervention.

Anthracyclines are widely used in cancer therapy, and have the highest risk of irreversible cardiotoxicity, over a longer period of time, than any other anticancer agent. Anthracyclines are also the most extensively studied agent in terms of cardiotoxic effects; the focus of this Review will, therefore, be on this class of drugs. We also provide suggestions on how the principles illustrated could be translated to reducing the cardiotoxicity of targeted therapies, particularly that of trastuzumab, for which potential cardiotoxicity is a major concern. We also describe a novel pathway algorithm for incorporating interdisciplinary, systems-based data into patient care. Integration and application of these approaches to clinical practice is, furthermore, proposed along with analysis of their cost-effectiveness in the prevention of cardiotoxicity.

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Anthracycline-induced cardiotoxicity

The incidence of heart failure after anthracycline use ranges from 3–30%, largely owing to differences in the patient populations studied.[3,5–7] However, this class of drugs remain a leading cause of cardiovascular morbidity and mortality after cancer treatment, particularly when used in combination with chest radiation therapy.[8] The delayed manifestation of chronic cardiotoxicity is a significant challenge to patient management, as many patients might not receive regular preventive surveillance or management at the time that symptoms develop. Thus, a growing number of survivors of cancer are at an increased lifetime risk of anthracycline-induced cardiotoxicity (currently >5 million in the USA alone), although no reliable estimates are available to quantify this risk. Assessing the long-term risk of anthracycline-induced cardiotoxicity before committing a patient to anthracycline therapy would therefore be of great value.

Key points

- Findings from 'systems biology' approaches that merge technology from life sciences, engineering, computer science, natural sciences and mathematics have elucidated adverse drug effects from a cellular to whole-organism level
- Systems biology approaches hold promise for chemotherapy-induced cardiotoxicity, which has received increasing attention following improvements in long-term survival of patients with cancer, resulting in the new discipline of cardio-oncology
- Patients receiving chemotherapy have marked inter-individual variations in cardiotoxicity risk beyond pre-existing cardiovascular disease and its risk factors, indicating an influence of genetic factors
- Genetic variants that predispose to anthracycline-induced cardiotoxicity, including genes involved in drug transport and metabolism, energy utilization, oxidative stress, apoptosis and cytoskeletal regulation are the best characterized genetic influences
- Incorporation of genetic signatures, protein biomarkers and mathematical models for the development of new risk prediction tools and therapeutic targets could reduce the extent of chemotherapy induced cardiotoxicity
- Defining patients' specific predispositions, in order to individualize treatment strategies and thus yield the greatest treatment benefits at the lowest possible cardiotoxicity risk, is the ultimate goal of cardio-oncology

Unlike chronic cardiotoxicity, where cumulative dose of anthracyclines is the most robust predictor of adverse effects, acute anthracycline-induced cardiotoxicity rarely constitutes a clinical challenge, presenting primarily as electrocardiographic changes and/or arrhythmias.[2,5,6,9] Findings of a meta-analysis published in 2013 confirmed the cumulative dose of anthracyclines as the most robust predictor of cardiotoxicity.[10] Given that anthracyclines are administered with dose adjustments (often based upon the individual judgement of the clinician), to maximum cumulative doses of 400–550 mg/m^2 of doxorubicin, the approach to prevention of cardiotoxicities has been one of 'gestalt'. At high doses of doxorubicin, the risk of heart failure increases exponentially, as demonstrated by the findings of several cohort studies[2,7,11–14] (Supplementary Figure 1). Some patients, however, are seemingly able to tolerate these, and even higher doses without any untoward cardiac consequences, whereas other patients cannot endure even half of these outlined threshold doses without cardiotoxicities.

From a mechanistic standpoint, the cellular targets of doxorubicin are topoisomerase II α (TOP2A) and topoisomerase II β (TOP2B), which are nearly 70% identical in structure and are integral to DNA transcription, replication and recombination.[15–17] TOP2A is highly expressed in rapidly proliferating tissues, such as tumour cells, but is not detected in adult mammalian cardiomyocytes.[18–20] TOP2B is expressed in cardiomyocytes,[19,21] and mediates anthracycline-induced cardiomyopathy.[22,23] TOP2B forms a ternary complex with doxorubicin and DNA, triggering cell death by various pathways (Figures 1 and 2).[22–29]

Systems-oriented approaches

Systems biology approaches combine the fields of biology with engineering, physics, mathematics and computational science to understand the collective behaviour of the cells, organs and the whole organism.[30,31] These approaches address the sequelae of interacting biological components, from the molecular and intracellular network level, through to the cellular, tissue and organ level and can even include incorporation of clinical indicators and outcome analyses. Such approaches are necessary to understand the complexity of adverse drug effects that take place at the cellular level, but are only observed clinically in patients.

Genomic variants in candidate-gene studies

The cumulative dose of doxorubicin administered to patients during cytotoxic therapy is generally limited to <400 mg/m^2, although, a wide interindividual susceptibility to cardiotoxicity is still observed in some patients at submaximal doses.[18,32] Conversely, other patients tolerate doxorubicin doses that vastly exceed 400 mg/m^2.[33] Why some individuals are especially sensitive to the cardiotoxic effects of anthracyclines, and how to identify individuals who are more susceptible to drug-induced oxidative damage and apoptosis before commiting to this therapy is currently unclear.[18] Genomic variations, likely coupled with environmental factors, might influence intracellular drug concentrations and explain this inter-individual variation in both effectiveness and safety of doxorubicin.[34]

Genomic variations that might explain the differences in response to doxorubicin can be assessed in candidate gene studies, which aim to identify associations between preselected genes with anticipated biological functions and relevant disease phenotypes. A case-control study design is typically used to determine differences in the frequency of the genetic variations between individuals with the disease and healthy individuals. Despite the established cardiotoxicities associated with anthracyclines, genomic variants that could influence doxorubicin toxicity have only started to be more thoroughly investigated in patients in the past decade.[35,36] Several key genomic variants have been discovered by various groups investigating the pharmacogenomics of doxorubicin-induced cardiotoxicity (Figure 1 and Table 1).[35] Several of these reported genomic variants, which are proven to be associated with doxorubicin-induced cardiotoxicity, include genes that encode proteins involved in oxidation reactions, as well as DNA repair, transport and efflux pumps, such as multidrug resistance protein 1 (MDR1, encoded by *ABCB1*), multidrug resistance-associated protein 1 (MRP1, encoded by *ABCC1*), and canalicular multispecific organic anion transporter 1 (MRP2 encoded by *ABCC2*).[35–39]

ABCB1 expression can be increased by the presence of one of several genomic variants in the promoter regions, or an exon 21 polymorphism, which results in a cardioprotective effect and confers resistance to anthracyclines[40–42] (Table 1), in addition to many other drugs. Genomic variants in *ABCC1* and *ABCC2* are associated with doxorubicin-induced cardiotoxicity (Table 1).[37,43] The common *ABCC1* genomic variant Gly671Val leads to increased doxorubicin-induced cardiotoxicity, while the variant Arg433Ser results in substantially lower levels of intracellular doxorubicin (~20%) *in vitro*; inhibition of MRP1 worsens cardiotoxicity owing to increased intracellular doxorubicin concentrations, as outlined in experimental[34,37,44] and clinical[37,44] studies (Table 1).

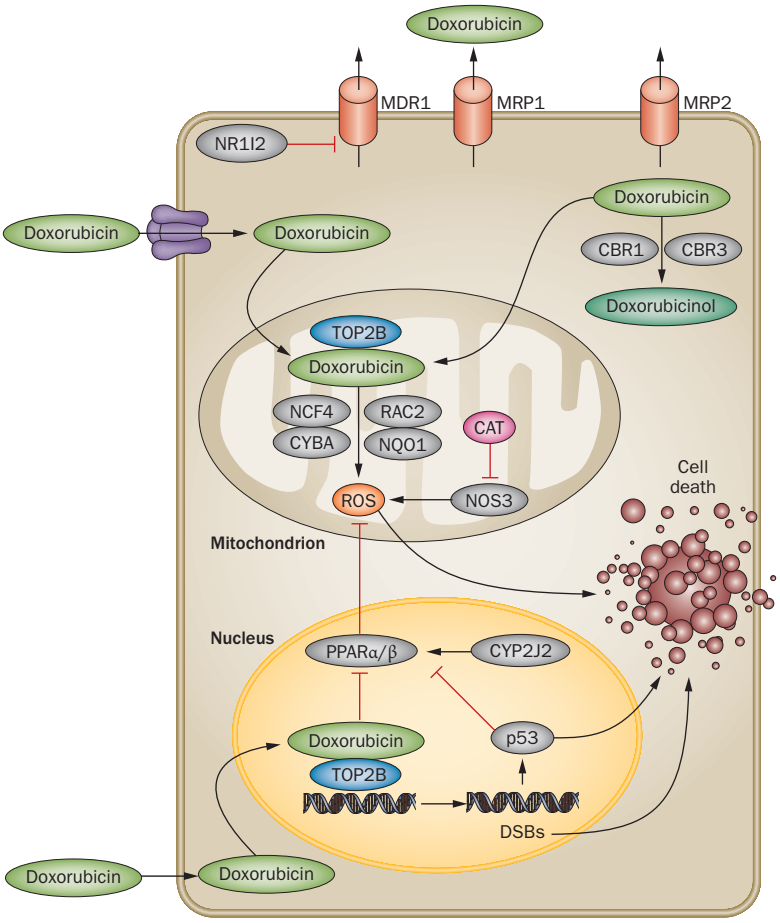

Figure 1 | Candidate genes implicated in doxorubicin-induced cardiotoxicity. Genomic variants have been identified in several proteins involved in the cardiotoxicity signalling cascade triggered by doxorubicin. Genes encoding cell membrane proteins, such as doxorubicin transporters and efflux pumps include *ABCB1*, *ABCC1*, *ABCC2* and *SLC22A16*, and can cause toxicity if mutations affect transporter function. Candidate genes encoding gene transcription regulators, include *PPARα*, *PPARβ* and *TP53*. Both TP53 and the doxorubicin–TOP2B complex inhibit expression of *PPARα* and *PPARβ* by binding to their gene promoters.[23,48] *PPARα* and *PPARβ* are nuclear receptors and transcriptional co-activators, and are key regulators of oxidative phosphorylation and mitochondrial biogenesis involved in doxorubicin-induced cardiotoxicity.[23,47,49,50] Genes encoding mitochondrial proteins are critical for oxidative phosphorylation and generation of ATP and include *CAT, NOS3*, *NQO1*, *CYBA*, *NCF4* and *RAC2*, which form the NAD(P)H oxidase complex. Genomic variants in these proteins contribute to mitochondrial dysfunction, thereby increasing reactive oxygen species (ROS) generation, which (along with apoptotic factors) ultimately culminates in cardiomyocyte death.[23] Proteins often found in the cytosol include CBR1 and CBR3 that metabolize doxorubicin to doxorubicinol, and NR1I2 that inhibits MDR1. Abbreviations: ABCB1, ATP-binding cassette, sub-family B (MDR/TAP), member 1; ABCC1, ATP-binding cassette, sub-family C (CFTR/MRP), member 1; ABCC2, ATP-binding cassette, sub-family C (CFTR/MRP), member 2; CAT, catalase; CBR1, carbonyl reductase 1; CBR3, carbonyl reductase 3; CYBA, cytochrome B558 α subunit; CYP2J2, cytochrome P450 2J2; DSB, DNA double-stranded breaks; GRIN1, glutamate receptor, ionotropic, N-methyl D-aspartate 1; GRIN2D, glutamate receptor, ionotropic, N-methyl D-aspartate 2D; MDR1, multidrug resistance protein 1; MRP1, multidrug resistance-associated protein 1; MRP2, multidrug resistance-associated protein 2; NADPH, nicotinamide adenine dinucleotide phosphate oxidase; NCF4, neutrophil cytosolic factor 4, 40 kDa; NOS3, nitric oxide synthase 3; NQO1, NAD(P)H dehydrogenase, quinone 1; NR1I2, nuclear receptor subfamily 1 group I member 2; PPARα/β, peroxisome proliferator-activated receptor γ co-activator 1-α/β; RAC2, ras-related C3 botulinum toxin substrate 2 (rho family, small GTP-binding protein Rac2); ROS, reactive oxygen species; SLC22A16, solute carrier family 22 (organic cation/carnitine transporter) member 16; TOP2B, topoisomerase 2β; TP53, tumour protein p53.

Genome-wide association studies

Genomic variants can also be studied in investigations using GWAS. Case-control studies using GWAS enable simultaneous examination of several common genetic variants or detection of single nucleotide polymorphisms (SNPs). 1p32.1 has been preliminarily identified as a novel genomic locus for anthracycline-induced cardiotoxicity.[45] Prior to this finding, results of one study demonstrated that overexpression of a nearby gene, cytochrome P450 family 2 subfamily J polypeptide 2 (*CYP2J2*), is cardioprotective (Table 1).[46] CYP2J2 is an epoxygenase that activates the nuclear receptor and transcriptional co-activator peroxisome proliferator-activated receptor-γ co-activator 1-α (PPARα; PGC-1α).[47] Of note, expression of the PPARα gene is downregulated in mice with doxorubicin-induced cardiotoxicity compared with mice not exposed to doxorubicin (Figure 1).[23,47] This finding suggests that activation of PPARα by CYP2J2 could potentially contribute to this cardioprotective effect. Further studies should, therefore, be pursued in order to identify and/or confirm the effects of cardiotoxic genomic variants of *CYP2J2* that might be associated with the 1p32.1 region.

Genomic profiling

Microarray studies using cardiomyocyte-specific *TOP2B*$^{-/-}$ knockout mice with doxorubicin-induced cardiomyopathy have revealed activation of genes in the tumour-suppressor protein TP53 and β-adrenergic signalling pathways preceding the repression of mitochondrial function and oxidative phosphorylation pathways (including PPARα and PPARβ; PGC-1β).[23] Both the TP53 and the doxorubicin–TOP2B complex can inhibit expression of *PPARA* and *PPARB* by binding to their genes' promoters (Figures 1 and 2).[23,48] PPARα and PPARβ are key regulators of mitochondrial biogenesis in heart failure.[49,50] Expression of superoxide dismutase, an antioxidant enzyme, was also downregulated in cardiomyocyte-specific knockout *TOP2B*$^{-/-}$ mice with doxorubicin-induced cardiotoxicity,[23] and this effect was likely mediated by PPARα.[51] Taken together, results from these studies suggest that doxorubicin binds TOP2B, disrupts the transcriptome and represses expression of *PPARα* and other genes that are integral to nuclear and mitochondrial function, thereby releasing inhibition of superoxide dismutase and increasing reactive oxygen species (ROS) generation, which (along with apoptotic factors) ultimately culminates in cardiomyocyte death.[23]

Findings of a genomic profiling study also identified a greater than twofold reduction in expression of >100 genes, such as T-cell leukaemia/lymphoma 1A (*TCL1A*) and *ABCB1* in women with anthracycline-induced cardiomyopathy.[18] The TCL1A protein co-activates AKT1, a major pro-survival factor that is protective against doxorubicin-induced cardiomyopathy (Figure 3).[52] The *ABCB1* transcript encodes the transporter ATP-binding cassette subfamily B member 1, which is an efflux transporter protein that pumps doxorubicin out of the cardiomyocyte (Figure 1).[53]

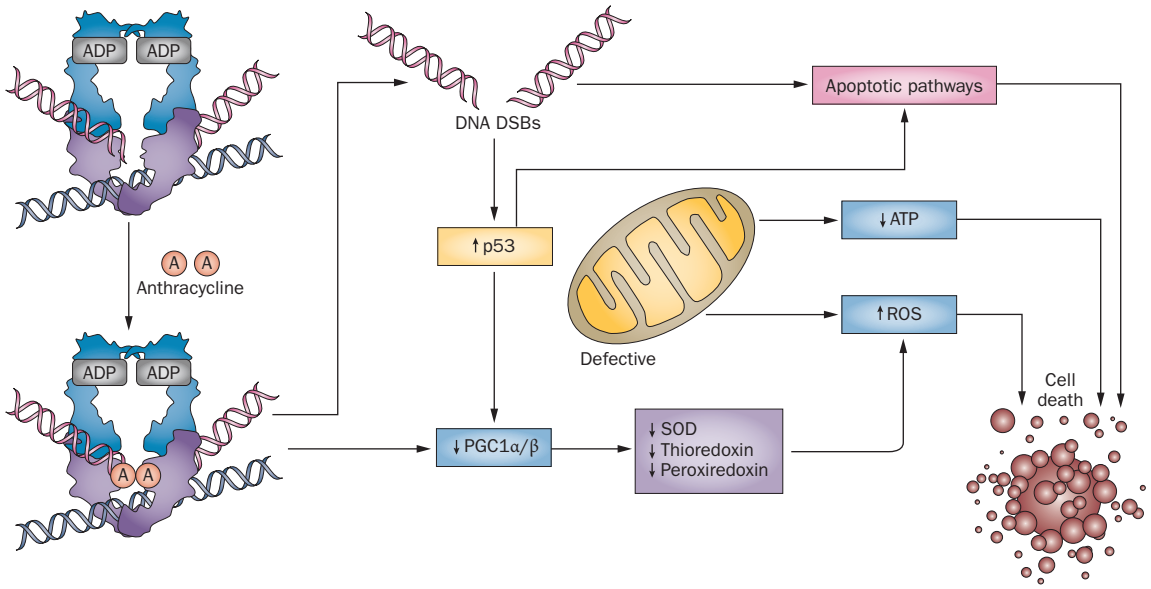

Figure 2 | Doxorubicin-induced cardiotoxicity is mediated by TOP2B. Doxorubicin induces cardiotoxic changes within the cell, which are mediated by TOP2B, including double-stranded breaks, generation of reactive oxygen species, disruption of mitochondrial biogenesis, and activation of apoptosis, via the p53 pathway. Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; DSB, DNA double-strand break; ROS, reactive oxygen species; SOD, superoxide dismutase; TOP2B, DNA topoisomerase 2β. Reproduced with permission obtained from John Wiley and Sons © Vejpongsa, P. & Yeh, E. T. *Clin. Phamacol. Ther.* **95**, 45–52 (2015).

Efflux proteins are energy-dependent plasma membrane transporters that remove a variety of intracellular agents, including anthracyclines.[54,55] Wild-type efflux transporters, including MDR1 and MRP1, in addition to low and very low levels of multidrug resistance-associated protein 3 (MRP3) and MRP2, respectively, are expressed in human cardiomyocytes, and together clear the cell of about 50% of intracellular doxorubicin.[56] The expression level of the these efflux transporters is variable; for example, administration of doxorubicin can lead to a 10-fold increase in *ABCB1* expression.[9] By contrast, decreased blood transcript levels of *ABCB1* were noted in women with a decline in cardiac function following doxorubicin chemotherapy along with decreased levels of *TCL1A* transcripts, which is noteworthy as *TCL1A* co-activates AKT, and AKT and PI3K signalling modulate *ABCB1* expression (Figure 3).[18] Suppressed *ABCB1* expression leads to abnormally elevated intracellular levels of doxorubicin and subsequent cardiotoxicity, as supported by the findings of various studies including *in vitro* experiments using verapamil to inhibit MDR1.[18,57–60] If cardiomyocytes undergo similar changes in gene regulation as peripheral blood monocytes undergo in response to anthracyclines,[61] then the observed decreased expression of *ABCB1* and multidrug resistance protein 3 (*ABCB4*) would lead to intracellular accumulation of anthracyclines, resulting in mitochondrial dysfunction, generation of reactive oxygen species, DNA damage and apoptosis (Figure 1).[18]

Proteomics

Integrative proteomics has been used to study anthracycline-induced cardiotoxicity in rabbits.[62] Alterations in protein expression largely involve proteins that are responsible for oxidative phosphorylation, energy channeling, antioxidant defense, mitochondrial stress, proteolysis and apoptosis.[62] Significant changes were also noted in the expression of basement membrane and extracellular matrix proteins.[62] These findings provided a glimpse into the complex proteomic signature of anthracycline-induced cardiomyopathy.

The mouse heart proteome was also analysed after chronic anthracycline exposure.[63] Alteration in the expression of 52 proteins was noted, with the vast majority involved in energy metabolism. Altered energy metabolism, along with production of ROS, impairs cytoplasmic calcium homeostasis and interrupts the expression of cytoskeletal proteins, thereby contributing to contractile dysfunction and adversely affecting the haemodynamics of the left ventricle.[63]

Mathematical and computational modelling

A mathematical model has been created to predict the risk of doxorubicin-induced cardiomyopathy (Supplementary Figure 1).[14] Accordingly, the risk of developing cardiotoxicity is can be calculated as a function of the number of cycles of cytotoxic therapy and a correction factor (coefficient) that assumes administration of a 50 mg/m^2 dose of doxorubicin. The mathematical curve fits experimental data from two studies[11,12] and suggests that cardiotoxicity is a quadratic function of cumulative doxorubicin dose.

Another mathematical model, for comparing various modes of delivery of doxorubicin, has also been developed.[64] Bolus injection, continuous doxorubicin infusions of various durations and liposomal and

Table 1 | Doxorubicin-induced cardiotoxicity-related pharmacogenetic variants

Gene	rs	Mutation	Variant	Biological process	Cardiotoxicity phenotype*
ABCB1	rs1128503	1236C>T	T	Drug transport	Increased drug exposure, reduced clearance
	rs35657960	Leu662Arg	G	Drug transport	Incresed resistance *in vitro*
	rs35730308	Trp1108Arg	C	Drug transport	Reduced resistance *in vitro*
ABCC1	rs45511401	Gly671Val	T	Drug transport	Cardiotoxicity
	rs60782127	Arg433Ser	T	Drug transport	Increased resistance *in vitro*
	rs4148356	Arg723Gln	A	Drug transport	Reduced resistance *in vitro*
ABCC2	rs17222723	Val956Glu	A	Drug transport	Acute cardiotoxicity
CAT	rs10836235	c.66+78C>T	T	Oxidative stress	Cardiotoxicity
CBR1	rs1143663	Val88Ile	A	Drug metabolism	Reduced activity *in vitro*
CBR3	rs8133052	Cys4Tyr	A	Drug metabolism	Lower ratio of doxorubicinol plasma AUC/doxorubicin AUC *in vivo*; higher V_{max}, lower Km *in vitro*
CYBA	rs4673	Tyr72His	T	Oxidative stress	Acute cardiotoxicity, inferior event-free survival
NCF4	rs1883112	−212G>A	A	Oxidative stress	Chronic cardiotoxicity
NOS3	rs1799983	Asp298Glu	G	Oxidative stress	Increased risk of cancer recurrence after doxorubicin therapy
NQO1	rs1800566	Pro149Ser	A	Energy utilization	Very low activity *in vitro*
NR1I2	NA	PXR*1B, 2654T>C	C	Regulation of drug metabolism and/or transport and apoptosis	Lower doxorubicin clearance
RAC2	rs13058338	7508T>A	A	Energy utilization	Acute cardiotoxicity
SLC22A16	rs714368	His49Arg	C	Drug transport	Increased drug exposure
TOP2A	NA	Amplification	Multiple	DNA regulation	Increased response

*Refers to performance *in vivo* unless otherwise stated. Abbreviations: AUC, area under the concentration-time curve; NA, not available; rs, reference single-nucleotide polymorphism cluster number.

thermoliposomal delivery are included, and experimental data regarding the rate and saturability of cellular uptake are simulated. The model predicts peak tumour cell concentrations as a measure of antitumour effectiveness. Moreover, findings of this analysis indicate that IV infusions of higher doses of doxorubicin, for longer durations, are protective against cardiotoxicity, and therefore, longer durations of administration are superior to other protocols.[64] Use of liposomal drug delivery systems for delivery of doxorubicin might also prove to be protective against cardiotoxicity if the drug is released at an optimal rate.[64,65] Use of thermosensitive liposomes might offer an advantage over more-traditional drug-delivery methods as locally applied temperature control using a combination of applicators steering the heating focus and power regionally, and locally, enable external control of drug release to the tumour region in a more potent and specifically targeted manner.[65,66]

Use of another computational tool has demonstrated that superior antitumour effects can be achieved using delivery of thermosensitive liposomes relative to continuous infusion over 2 h, and that the localization afforded by thermosensitive drug delivery leads to lower drug concentrations in normal tissues.[67] Other models have been useful in aiding our understanding of doxorubicin pharmacokinetics and drug delivery, as well as its effects on cellular biology and function.[68–70]

Trastuzumab-induced cardiotoxicity

Genomic variants

The HER2 proto-oncogene is a member of the EGFR family (Figure 3).[71] HER2 is overexpressed in up to 25% of breast cancers in women, and is associated with a poor prognosis compared with that of patients with breast cancer who do not overexpress HER2.[72] In addition to cytotoxic therapy, these breast cancers are also treated with trastuzumab, which significantly improves disease-free and overall survival, although with an increased risk of cardiotoxicity.[73–80] A decrease in cardiac systolic function is the main measure of this risk, while severe heart failure and cardiac death remain rare clinical events (<1% and <0.5% of patients, respectively, in randomized controlled trials).[81,82] Intriguingly, the cardiotoxicity risk increases in patients who have the Ile655Val heterozygous *HER2* polymorphism, which is observed in as many as one third of patients with HER2-overexpressing tumours who are treated with trastuzumab.[83–85]

Synergy with anthracycline cardiotoxicity

In the clinic, susceptibility to doxorubicin-induced cardiotoxicity worsens with concomitant, or subsequent use of trastuzumab (and occurs in as few as 2–5% to >20% of patients), and is partially reversible with discontinuation of trastuzumab.[75,78,82,86,87] *HER2* conditional knockout adult mice generally develop

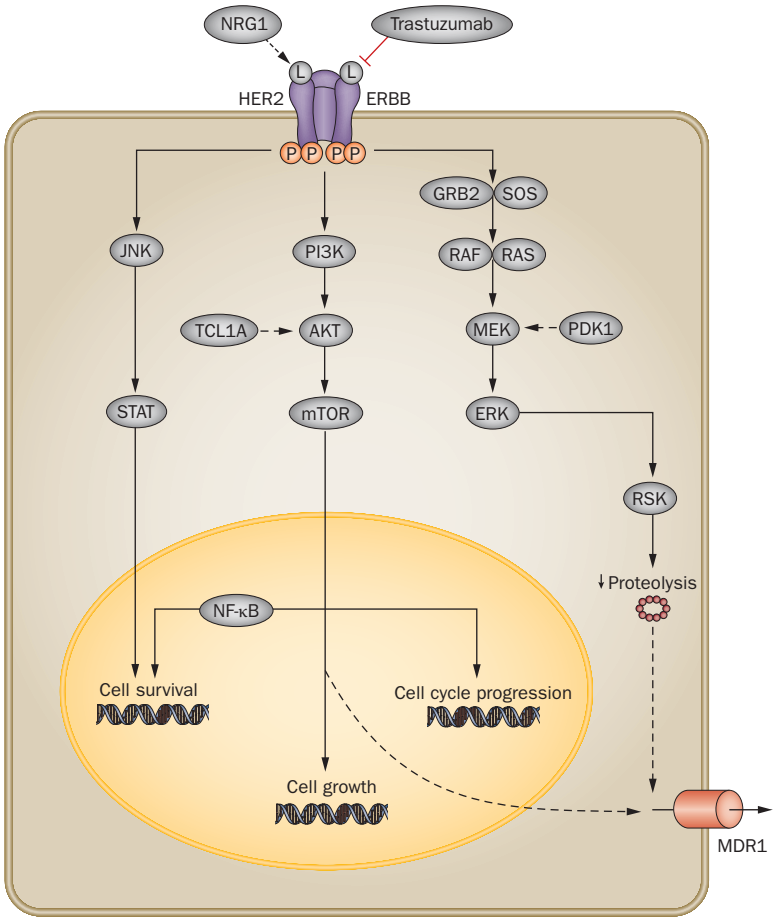

Figure 3 | Overlapping tyrosine kinase signalling pathways in anthracycline-induced toxicity. EGFR or ERBB family members are tyrosine kinases that dimerize when bound by ligand (L), which increases phosphorylation (P) to concomitantly activate the PI3K–AKT–mTOR, JNK–STAT and MAP kinase pathways, leading to gene transcription, cell proliferation, and survival. Ligands include NRG1, EGF, TGFα and FGF. For highly proliferative cells, such as malignant tumours, activation of these pathways also leads to angiogenesis, migration, adhesion and invasion. Trastuzumab, a targeted agent, inhibits HER2, which is localized specifically to transverse tubules (t-tubules) of cardiac ventricular myocytes, thus HER2 is an important contributor to cardiac cell function at the main site of excitation-contraction coupling in the cell.[89,149–151] Activation of the MAP kinase pathway decreases proteolysis of MDR1. Solid arrows indicate processes/pathways, and dashed arrows indicate activation of the destination protein by the origin protein. Abbreviations: GRB2, growth factor receptor-bound protein 2; MDR1, multidrug resistance protein 1; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor kappa B; NRG1, neuregulin-1; SOS, guanine nucleotide exchange factor; STAT, signal transducers and activators of transcription; TCL1A, T-cell leukemia/lymphoma 1A.

severe dilated cardiomyopathy, with a 50% reduction in the contractile function of the heart.[88,89] Thus, HER2, the preferred dimerization partner for all other ERBB receptors, particularly ERBB4 in cardiomyocytes, is thought to have a variety of roles in the normal physiology of the heart including morphology, hypertrophic growth, excitation–contraction coupling and survival.[90–92]

Inhibition of HER2 signalling with trastuzumab also antagonizes the effects of neuregulin 1 (NRG1), a member of the EGF family. NRG1 is a ligand for HER2 in heterodimers along with other members of the ERBB family in cardiac and skeletal muscle. NRG1 normally protects cardiomyocytes against stress by regulating ROS-induced cardiomyocyte apoptosis, inducing cell-cycle activity, promoting regeneration and improving cardiac function and survival.[93–96] This notion is supported by results from studies in which heterozygous knockout of NRG1 or ventricular-restricted deletion of *ERBB2* in mice exacerbates doxorubicin-induced cardiotoxicity.[88,97]

Trastuzumab prolongs disease-free survival in most patients, but also interferes with myocardial homeostasis and exacerbates doxorubicin-dependent signalling pathways in cardiomyocytes.[98,99] Patients receiving anthracycline monotherapy and rats with chronic doxorubicin-induced injury have transient upregulation of left ventricular expression of *ERBB2*, presumably representing a compensatory system intended to counteract oxidative stress and myocardial disarray.[99–101] Disruption of homeostasis provides a plausible mechanism to explain the detrimental synergy of ROS-induced cardiotoxicity and dilated cardiomyopathy caused by doxorubicin, in the setting of concurrent or sequential suppression of the ERBB/HER2 signalling pathway.[99,102] This disruption is consistent with the higher levels of myocardial oxidative stress observed in mice treated with doxorubicin and trastuzumab, and higher levels of myofibrillar disarray in rats treated with doxorubicin and a monoclonal anti-ERBB2 antibody than in mice or rats treated with doxorubicin alone.[99,103] Notably, neither trastuzumab nor NRG1 alone has any effect on myocardial oxidative stress or myofibrillar disarray under wild-type conditions.[99,103]

The increased sensitivity of trastuzumab-treated cardiomyocytes to doxorubicin-induced cardiotoxicity might also be mediated by MDR1. Suppression of MDR1 levels (via proteolysis) by inhibition of the MEK–ERK–RSK pathway (MAP kinase cascade) diminishes cellular resistance to antitumour agents, and, conversely, increases susceptibility to doxorubicin-induced cardiotoxicity (Figure 3).[54,104]

Clinical implementation

Systems medicine applied to cardio-oncology

Applying systems biology approaches to clinical problems defines 'systems medicine', and has great potential to advance current knowledge of personalized medicine and translational medicine.[105–109] A number of large academic hospitals have applied systems medicine approaches to the clinical implementation of pharmacogenomics[110–114] and for the avoidance of the adverse events typically experienced by some patients in response to various commonly used pharmacotherapies, including statins and warfarin.[115,116] Systems medicine approaches can also be applied to cardio-oncology.

Underdosing patients with drugs usually leads to ineffective therapy, while overdosing usually leads to toxicity.[117] Identification of individuals who are particularly prone to anthracycline-induced cardiotoxicity, for example, holds the key to tipping the balance between effective cytotoxic therapy and potentially life-threatening adverse effects.[18] These seemingly unpredictable or

Box 1 | Predictive power of *ABCB1* polymorphisms

- Presence of 2677G>T/A predicts a better prognosis in response to paclitaxel/carboplatin cytotoxic therapy in patients with minimal residual disease from ovarian cancer[130]
- Presence of 1236C>T predicts a better response to imatinib in patients with chronic myeloid leukaemia[131]
- Presence of 2677G>T/A and 3435C>T predicts a poor response to imatinib in patients with chronic myeloid leukaemia[131]
- Presence of 3435C>T predicts the rate of remission in patients with major depression receiving venlafaxine[154]
- Presence of 3435C>T predicts the escitalopram dose needed for remission in patients with major depression[154]
- Presence of 2677G>T/A, 1236C>T or 3435C>T individually predict higher short-term remission rates in patients with steroid-refractory ulcerative colitis treated with tacrolimus[126]

Abbreviation: *ABCB1*, ATP-binding cassette, sub-family B (MDR/TAP), member 1.

idiosyncratic adverse drug effects are likely genetic in origin, as elucidated by the identification of SNPs that are associated with significant inter-individual differences in drug clearance and toxicity.[34,118,119] Determining an optimal 'target window' for drug administration, based on inter-individual heterogeneity, might therefore, be necessary. Differences in pharmacokinetics are frequently caused by differences in relative gene and protein expression and/or function of efflux pumps and cytochrome P450 enzymes.[118] Genomic variants in such genes (for example, *ABCB1*) and also those involved in pharmacodynamics (such as *TCL1A*) could be assessed before treatment. Such pharmacogenomic assessments could be used to inform individualized treatment strategies and influence patient outcomes.

Integration of genomic variants

GWAS and SNP repositories, databases, and catalogues are currently being curated to facilitate collaborative inquiries into the functional implications of genomic loci for disease outcomes and pharmacological responses.[35,120–123] These initiatives will likely facilitate closer monitoring and earlier preventive interventions for patients with genomic variants that are associated with cardiotoxicity, such as the Gly671Val polymorphism in *ABCC1*.[34] The availability of such pharmacogenomic information could help establish genomic scoring systems to improve predictions of drug effectiveness and toxicities in individual patients.[124]

ABCB1 polymorphisms predict response

The presence of certain *ABCB1* polymorphisms currently can be used to predict a patient's response to various pharmacological agents, including chemotherapeutic drugs (Box 1). If validated in prospective clinical trials, pretreatment analysis of *ABCB1* polymorphisms might be used to pre-empt, predict and ultimately prevent the occurrence of toxicities (including from anthracyclines), while optimizing the effectiveness of therapies.

In *ABCB1*-injected *Xenopus laevis* oocytes, injection with the triple SNP variant 1236C>T, 2677G>T, 3435C>T had no functional consequences for the molecular transport of model substrates (such as imatinib) or inhibition by known inhibitors (such as verapamil, cyclosporine or dipyridamole).[125] This finding could reflect a net balanced (positive and negative) effect of simultaneous expression of all three alleles. Each allele, when expressed individually, might predict a better or worse prognosis; expressing all three alleles concurrently might thus mask the true effects of the individual alleles. Evidence from *Xenopus* oocyte models is supported by the finding that the presence of all three of these polymorphisms simultaneously did not predict the response to tacrolimus in patients with steroid-refractory ulcerative colitis (as opposed to the individual effects described in Box 1).[126] Molecular transport and inhibition by model inhibitors should, therefore, be investigated for use in model systems expressing single variants, to delineate the mechanisms underlying these observed phenotypes.

A multimarker model

Use of a multimarker model, comprised of multiple genetic variants (including an *ABCB1* variant) combined with clinical risk factors, enables successful prediction of anthracycline-induced cardiotoxicity in children.[127] Receiver operating characteristic (ROC) curves show that use of the combined model more accurately identifies patients who are at a higher risk of treatment-related toxicities (with an area under the curve of 0.87, specificity of 94.5% and sensitivity of 54.5%) than use of a genetic risk or clinical risk profile alone. Interactions among the studied variants, if any are in linkage disequilibrium, could possibly limit the sensitivity of multimarker models, although, investigators did attempt to mitigate against these possibly confounding effects of linkage in the statistical analysis.[128] This possibility should be taken into consideration when generating such models. Nevertheless, these promising results provide hope for the development of clinically useful risk scores in adults, which would incorporate measurements of both genetic risk factors along with measurements of clinical risk factors into a predictive risk model for heart failure in a general adult population. Such an approach increases the overall potential predictive power[43] of a clinical or genetic risk score, but this has not yet been evaluated in patients undergoing cytotoxic therapy. Perhaps such studies can be replicated for cytotoxic therapy-induced cardiotoxicity in adults, leading to improved risk prediction.[129]

Linkage disequilibrium and feedforward loops

When predicting the combined effect of multiple genomic variants, the influence of linkage disequilibrium and feedforward loops should be considered, as illustrated by the following. The *ABCB1* variants 1236C>T and 3435C>T have moderate linkage with each other (Figure 4), while the variants 2677G>T and 3435C>T also have moderate linkage disequilibrium. Each allele individually has an effect on

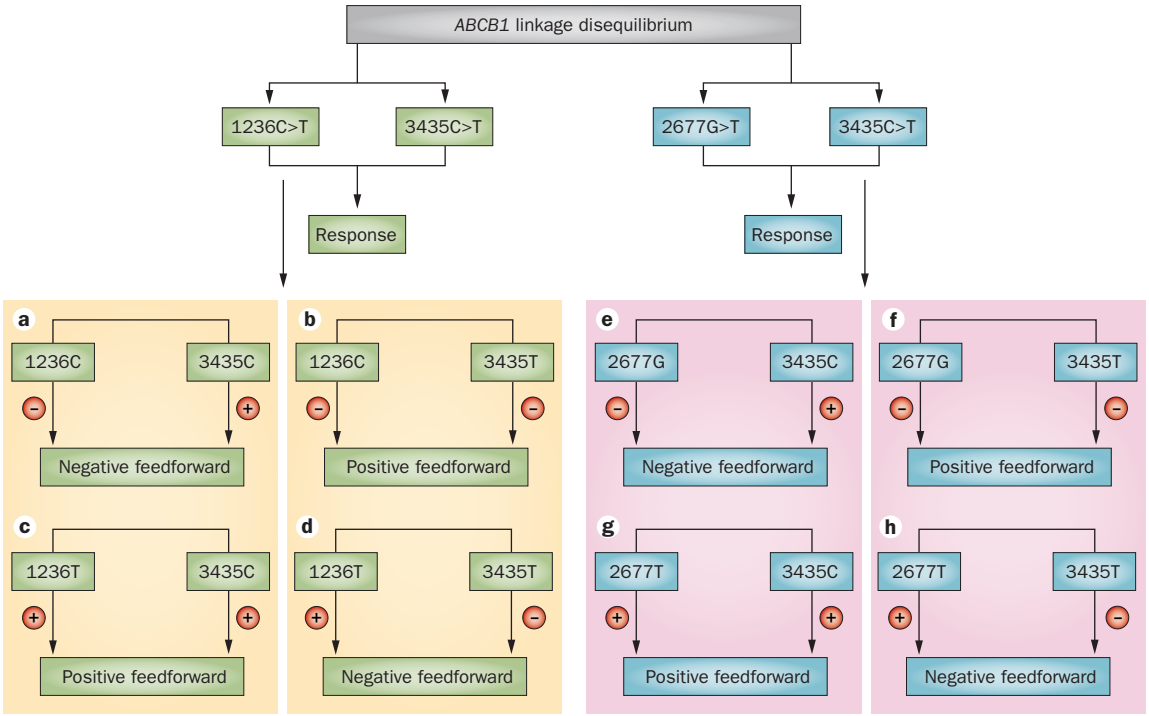

Figure 4 | *ABCB1* allele linkage disequilibrium feedforward loops. Several alleles of *ABCB1* exist in linkage disequilibrium, which can result in positive or negative feedforward loops. For example, the presence of 1236T and 2677T predicts better prognosis in response to imatinib in patients with chronic myeloid leukaemia, while the presence of 3435T predicts poor prognosis. For the 1236C>T/3435C>T loci two negative feedforwars loops (+/– [a] and +/– [d]) and two positive feedforward loops (+/+ [c] and –/– [b]) are possible at the linkage locus for 1236C>T and 3435C>T. Similarly, for the 2677G>T and 3435C>T loci two negative feedforward loops (+/– [e] and [h]) and two positive feedforward loops (+/+ [g] and –/– [f]) are possible at the linkage locus for 2677G>T and 3435C>T.[130,131]

pharmacotherapeutic responses.[130] For example, in patients with chronic myeloid leukaemia, who are receiving imatinib therapy, the presence of 1236C>T and 2677G>T predicts a better prognosis, whereas presence of 3435C>T predicts an inferior prognosis.[131] Differential effects might, therefore, result from a patient having certain combinations of these genomic variants. Two negative feedforward loops (+/– [1236C and 3435C] and [1236T and 3435T]) and two positive feedforward loops (+/+ [1236T and 3435C] and –/– [1236C and 3435T]) are possible at the 1236C>T and 3435C>T linkage loci, and a similar pattern can be observed at the 2677G>T and 3435C>T loci (Figure 4). This concept of linkage disequilibrium among cardiotoxicity-related alleles is not unique to *ABCB1*. Six *HER2* genomic variants exhibit strong linkage disequilibrium, implying that the functional clinical effect of each individual polymorphism might be diluted or augmented by the presence of any of the other variants.[83] The possibility of these effects will need to be considered when applying the effect of multiple genetic variants to clinical care.

A molecular switch is one possible outcome of feedforward (or feedback) loops. Conceptually, a molecular switch enables the (sometimes delayed) rapid conversion from one biochemical state to another. In the example of *ERBB2* genetic variation, if the outcome of linkage disequilibrium favours the Ile655Val polymorphism, this enables a switch between two molecular conformations.[132] Having the wild-type conformation of *ERBB2* confers a lower risk of cardiotoxicity than having the heterozygous form. A phenotypic switch also occurs when ERBB2 is bound by either trastuzumab or NRG1.[103] Binding of NRG1 to ERBB2 heterodimers activates the downstream MAPK and AKT pathways, which significantly reduces doxorubicin-induced myocardial disarray.[103] Conversely, binding of a monoclonal antibody to ERBB2 activates ERBB2 phosphorylation but without downstream activation of MAPK and AKT and results in a significant increase in doxorubicin-induced myocardial disarray.[103] This might account for the remarkable effects of trastuzumab on myocardial contractile dysfunction in the presence of doxorubicin.

Adventitious pharmacological inhibitors

Various commonly used therapies have been found to inhibit MDR1. These include verapamil, cyclosporine, dipyridamole, sertraline and paroxetine. Fluoxetine, citalopram and venlafaxine are weaker inhibitors of the wild-type form of this efflux pump.[125,128] Care should be taken when simultaneously treating patients for comorbidities requiring these medications. For example, in individuals simultaneously receiving treatment for breast cancer and depression or vasomotor instability, concomitant use of the listed antidepressants with doxorubicin cytotoxic therapy might have harmful unintended consequences, such as anthracycline-induced

Box 2 | The P*3 pathway, a suggested systems medicine-based approach

P1. Pre-empt: Incorporation of systems medicine data (such as *ABCB1* polymorphisms) into the EHR, along with epigenomic, environmental and clinical information to guide shared decision-making (Figure 4).

P2. Predict: Harnessing systems medicine data (such as individual patients' *ABCB1* polymorphisms) to determine personalized risk and predict the risk of cardiotoxicity (Figure 4).

P3. Prevent: Modification of the treatment regimen with cytotoxic dose adjustments and/or alternative drug delivery methods (such as heat sensitive liposomes), use of alternative cytotoxic medicines, and/or initiation of preventive cardioprotective medications while avoiding or altering the administration of additive or synergistic cardiotoxic therapeutics (such as trastuzumab, radiotherapy or verapamil).

Abbreviations: *ABCB1*, ATP-binding cassette, sub-family B (MDR/TAP), member 1; EHR, electronic health record.

cardiotoxicity. For this reason, use of alternative medications should be considered whenever possible in these patients. Similar attention should be given to any medications known, or suspected to interact with tyrosine kinases, MAPK signalling pathway components, or indeed any other signalling networks that are known to be involved in both cardiology and oncology.

Incorporation of genomic profiles

In addition to polymorphisms, prospective investigations are underway to determine whether pretreatment *ABCB1* expression or the level of MDR-like efflux activity, as well as the expression of various cardio-oncology network genes is also predictive of cardiomyopathy after cytotoxic therapy.[18] *TOP2B* gene expression is currently being investigated as a potential biomarker to help predict the risk of anthracycline-induced cardiotoxicity.[22] Screening of *TCL1A* levels has also been suggested as a means to identify women who are at a high risk of anthracycline-induced heart failure.[18] Links between levels of expression of other genes and potentially cardiotoxic outcomes should also be studied.

Assimilation of proteomic biomarkers

Serum protein levels, as reported in proteomics studies could also be explored as potential biomarkers of chemotherapy-related cardiotoxicity.[62,63] These might eventually become incorporated into the routine clinical monitoring of cardiotoxicity risks, as vigilance in clinical practice increases in this area. Increased assessment of biomarkers could also enable greater individualization in the use of cytotoxic therapy and guide the initiation of potentially cardioprotective therapies.

New therapeutic targets

Results from various systems-based methods would suggest that efflux pumps (MDR1, MRP1 and others), PPAR, TOP2B and NRG1, among other key players in the cardio-oncology signalling network, should be recognized as new targets for preventive drug therapy (Figures 1–3). For example, dexrazoxane is a bisdioxopiperazine compound that forms a complex with human TOP2B, bridging two TOP2B monomers in a close-clamp configuration, thereby preventing anthracycline binding to TOP2B.[133,134] Dexrazoxane is currently approved by the FDA, but only for patients with metastatic breast cancer who have already received 300 mg/m^2 of doxorubicin, and are considered to benefit from additional anthracycline therapy.[135] This restriction was generated following the concern that use of dexrazoxane has a risk of decreasing the effectiveness of cancer therapy at no perceived benefit in terms of prevention of heart failure with doses below this threshold. However, a decline in cardiac function precedes this clinical manifestation and starts at much lower cumulative doses; in fact, cardiac injury is induced by the first dose of anthracycline therapy.[4] Addressing these concerns by prevention of anthracycline-induced cardiotoxicity through use of dexrazoxane-induced TOP2B-degradation before initiation of cytotoxic therapy is currently being examined.[22]

Development of computational avatars

Cardio-oncology could benefit from the adaptation of computational and mathematical models of human cardiomyocytes. A generic standardized model could be modified with each patient's measured variables and genotypes (such as *ABCB1* and *ABCC1* polymorphisms, *TOP2B* and *TCLA1* gene-expression levels). Customized computational avatars could, therefore, be created for each individual patient.[136] Bolus injections, continuous drug infusions of various doses and durations and liposomal and thermoliposomal delivery methods could be simulated, as well as dose adjustments with provision of personalized recommendations. As such, dynamic systems and signalling networks that are already established can be individualized, modelled and perturbed to pre-empt, predict and prevent cardiotoxicity. Precision medicine involves a multidisciplinary approach, integrating basic science and clinical research with patient and outcomes information and, in this case, systems medicine data, with the ultimate goal of personalized systems data-based prescribing.[137–139] Indeed, computational models, described as molecular interaction maps, have been created to simulate cancer signalling pathways.[140] Results obtained with this model have been verified in experiments on human cell lines in response to various treatments, with reasonably accurate predictive validity.[140] A separate computational framework has also been developed, using machine learning and logistic regression mapped to clinical observations, drug target data, protein–protein interaction networks and gene ontology annotations to accurately predict cardiotoxicity (and other forms of adverse effects) of drugs tested in clinical trials.[141] This approach was also quite effective, with an area under the ROC curve of 0.77 and specificity of 78%. These findings demonstrate that adaptations, based upon findings from computational avatars and personalized models, could serve as clinical adjuncts in guiding precision medicine.

The P*3 pathway to systems medicine

The P*3 pathway for systems medicine (Box 2) is suggested as an organized algorithm that is intended to

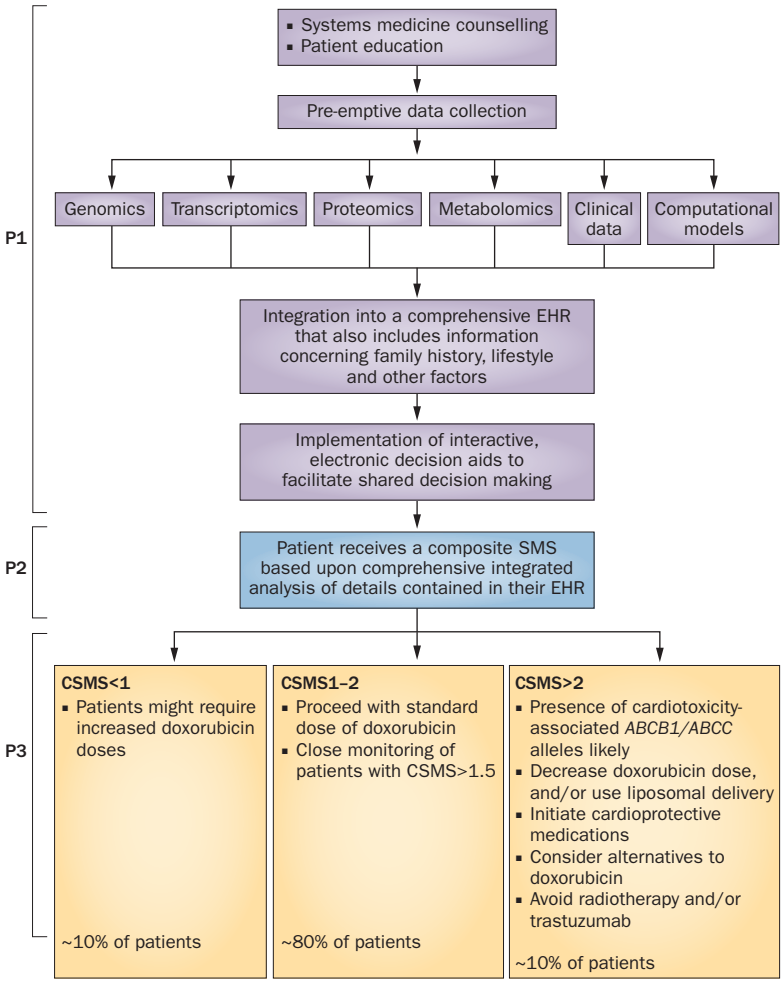

Figure 5 | The P*3 approach to systems medicine. The P*3 pathway introduces a method for incorporation of systems medicine data into clinical practice. Pre-emption (P1) is represented in purple. Systems medicine data should be integrated into the electronic medical or health record (EMR or EHR), along with clinical information and other demographics, including family history and environmental or lifestyle contributors. Interactive electronic decision aids should facilitate shared decision-making. Prediction (P2) is represented in blue and incorporates stratification mechanisms for risk prediction, such as what could be termed a CSMS, which incorporates clinical factors as well as systems medicine data. In the example of clinical cardio-oncology, the CSMS should be normalized to place individual patients into three risk categories (high, intermediate and low). Prevention (P3) is represented in yellow, and involves personalized prevention strategies that simultaneously optimize therapeutic efficacy and safety for the individual patient. Use of decision aids is useful in shared decision-making sessions with patients.[152] Pictograms could be used in shared decision-making to assist patients with difficult decisions regarding therapy modification, given a probabilistic cardiotoxicity risk based upon systems medicine data. Both pictograms and decision aids should minimize the burden on providers' expertise and resources for communicating information in the practice of systems medicine.[153] Abbreviations: CSMS, composite systems medicine score; EHR, electronic health record; SMS, systems medicine score.

guide global integration of output from systems-based approaches applied to preemption (P1), prediction (P2) and prevention (P3) into clinical medicine (Box 2, Figure 5). Several genomic variants have already been tested in routine oncology practice in order to help determine the choice of targeted therapy (for example, EGFR, KRAS and/or ABL kinase inhibitors),[142] although no variants have been investigated specifically for the purpose of pre-empting, predicting or preventing cardiotoxicity in patients receiving chemotherapy. Integration of data from a systems medicine approach into patients' electronic medical or health records will likely provide a foundation for implementation into clinical practice.[109,114,136,143] With pre-emptive information readily available electronically to the practitioner,[114] real-time decisions can be made to enable modification of the approach to therapy, thus avoiding cardiotoxicity.

Therapy modification in P*3 (Figure 5) for cardiotoxicity prevention would include initiation of medications such as dexrazoxane, angiotensin-converting-enzyme (ACE) inhibitors, β-blockers, angiotensin receptor blockers, statins and/or institution of an exercise programme (with positive effects potentially mediated by NRG1[91]).[3,144,145] Genetic or systems medicine counselling should also be incorporated into pretreatment consultations with patients to help inform treatment choices.[136] In the future, such counseling will likely include addressing a potential role of pharmacogenomics and use of other systems medicine data in predicting responses to protective drugs, such as ACE inhibitors and β-blockers, which are often used in patients with cardio-oncology related-health problems (Figure 5). Before such information can be incorporated into routine clinical practice, however, answers to a variety of questions would need to be provided. Such questions include why many patients with cardiomyopathy derive a benefit from these medications, yet other patients continue to deteriorate, and what accounts for this differential response. A striking difference in outcomes between two apparent populations exists in the survival curve of patients who develop doxorubicin-induced cardiomyopathy[146]—responders and nonresponders—are these two populations genetically different? Integration of related systems medicine data into patients' electronic health record as part of the P*3 pathway would be an important first step towards answering these questions.

With this process in mind, we recommend use of the P*3 pathway to optimize decision-making in systems medicine, in this case in the context of cardio-oncology. This outlined pathway might help ease the incorporation of risk-score calculations into prevention and treatment guidelines for clinical practice, which has so far proven to be elusive.[147] This pathway can also be used in conjunction with a formal risk-benefit framework for refining the translation of systems data into clinical practice.[148]

Economic considerations

In order for systems medicine approaches to cardio-oncology to have any clinical utility, testing will need to be widely available, accessible and cost-effective. In the absence of any formal investigations in this area, we present two scenarios that involve a patient with a high risk of cardiotoxicity. Scenario A involves one genomic array test, along with 6 months of generic ACE inhibitor, statin and β-blocker use, and dexrazoxane before each administration of 15 courses of doxorubicin. Scenario B involves 10 years of heart failure-related hospitalizations

and generic ACE inhibitor and β-blocker use, along with outpatient intracardiac defibrillator placement. An obvious conclusion would be that a total estimated cost of US$16,000 in scenario A for a prophylactically treated patient found to be at a high risk of cardiotoxicity is more cost-effective than a total estimated (minimum) cost of $100,000 in scenario B for an untested and untreated patient with a high cardiotoxicity risk who develops doxorubicin-induced cardiomyopathy. The marked potential cost savings ($84,000) associated with scenario A warrant further investigation through formal economic analyses, including life expectancy and quality-of-life data for adjusted patient life years.

Beyond these two simplified scenarios, additional mechanisms are involved in patient testing and selection for pre-emption, prediction and prevention of cardiotoxicities. In order to minimize costs, custom pharmacogenomics arrays and standardized genotyping infrastructure,[109,123] as well as centralized proteomics and mathematical and computational modelling core facilities will need to be established. Patient selection and phenotypic risk models will also need to be developed,[109] along with genomic risk scores and simplified but comprehensive predictive algorithms, thus avoiding the use of blind screening. Consolidated interactive didactic modules and customized training sessions will also be required in order to enhance both health care professionals' and patients' systems medicine and genomic literacy.[149] Once core Clinical Laboratory Improvement Amendments (CLIA)-certified sites are instituted and their services are made broadly available, with central repositories, this will enable efficient use of a wealth of systems medicine data that can be leveraged for personalized patient care. Use of computational avatars will also become more economically viable once computational modelling in systems medicine becomes more ubiquitous. These changes will likely occur in much the same way as sequencing an entire human genome now costs approximately $1,000, whereas the cost approached $400 million >10 years ago.[150] Perhaps within the next decade, integration of systems medicine into patient electronic health records will further the use of precision medicine, which has been hailed by the NIH as an exceptional opportunity in medical science.[150] Parsimonious methods will be required to ensure equitable access, that improves the health of individuals on a global level. In fact, suggestions have been made that systems approaches could also be applied to complex health-care systems beyond biological data in living organisms, with extension to finances, quality and healthcare policy.[109]

Conclusions

Cytotoxic therapy-induced cardiovascular toxicity is a life-threatening and multifactorial adverse effect that might benefit from systems-oriented approaches to better understand the pathways involved. This complexity has inspired the use of tools in systems biology to study the expression and function of the entire genome. Genomic profiling has identified TOP2B as an essential driver of doxorubicin-induced cardiomyopathy. Genetic variants have been discovered using GWAS and candidate-gene studies of the efflux pumps *ABCB1*, *ABCC1* and *ABCC2*, and for genes that encode other proteins such as *ERBB2*. These available data could add value to smaller, shorter, cheaper and individualized clinical trials designed to determine the effectiveness and safety of precision patient care.[30] The introduction of systems biology methods, including mathematical and computational models, will likely improve the assessment of patient prognosis in these trials. This approach will help improve drug effectiveness at lower doses and avoid toxicity at higher doses. Investigations continue to yield systems medicine data, and we approach an era during which individualized systems-based approaches, aimed at optimizing personalized treatment, might feasibly be used as a prescribing tool in clinical practice (Figure 4). The P*3 pathway, and other algorithms might be useful in charting a path forward in systems medicine, particularly if applied to cardio-oncology. Use of such a tool, which leverages systems medicine for prevention and therapy, might also limit the occurrence of post-marketing drug withdrawal owing to adverse drug effects,[137] and accelerate adequate treatment and cure.

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Author contributions

S.-A.B. and J.H. researched data for this article, all authors made a substantial contribution to discussions of content, S.-A.B. and J.H. wrote the manuscript, and all authors made a substantial contribution to editing and reviewing the manuscript before submission.

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