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Concise Review: Precision Matchmaking: Induced Pluripotent Stem Cells Meet Cardio-Oncology

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ABSTRACT

As common chemotherapeutic agents are associated with an increased risk of acute and chronic cardiovascular complications, a new clinical discipline, cardio-oncology, has recently emerged. At the same time, the development of preclinical human stem cell-derived cardiovascular models holds promise as a more faithful platform to predict the cardiovascular toxicity of common cancer therapies and advance our understanding of the underlying mechanisms contributing to the cardiotoxicity. In this article, we review the recent advances in preclinical cancer-related cardiotoxicity testing, focusing on new technologies, such as human induced pluripotent stem cell-derived cardiomyocytes and tissue engineering. We further discuss some of the limitations of these technologies and present future directions. STEM CELLS TRANSLATIONAL MEDICINE *2019;8:758–767*

SIGNIFICANCE STATEMENT

Many chemotherapeutic agents cause acute and chronic cardiovascular complications. The development of rigorous preclinical models is necessary to predict human cardiotoxicity and elucidate the underlying mechanisms of cardiotoxicity.

INTRODUCTION

Several common chemotherapeutic agents, including anthracyclines, alkylating agents, antimetabolites, antimicrotubule agents, tyrosine kinase inhibitors (TKIs), and proteasome inhibitors (PIs) are associated with an increased risk of acute and chronic cardiovascular complications [1]. Current preclinical strategies for predicting cardiotoxicities are inadequate. There is a pressing need for the development of relevant preclinical models to predict human cardiotoxicity and to elucidate the underlying mechanisms contributing to the cardiotoxicity of common oncology therapies.

The objective of this review is to highlight recent advances in preclinical cardiotoxicity testing *in vitro* with an emphasis on human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) and tissue engineering approaches. These new technologies promise a revolutionary *in vitro* model that can improve cardiotoxicity assessment toward precision medicine.

CARDIO-ONCOLOGY: A RAPIDLY EMERGING FIELD

The National Cancer Institute estimates that there is a ~40% lifetime risk of developing cancer in the U.S. [2]. Anticancer therapies have dramatically improved the outcomes of cancer treatment over the past decades and the overall cancer death rate has declined by almost 25% since 1990 [2]. The demand for cardio-oncology services grows along with increasing cancer survivorship rates. However, cardiotoxicity-related adverse effects caused by these anticancer therapies are on the rise. The incidence of cardiotoxicity differs greatly between chemotherapeutic agents, with pre-existing cardiovascular disease and other risk factors playing an important role in the development of cardiomyopathy secondary to cancer treatment. Reported incidences of chemotherapy-induced cardiotoxicity vary based on how cardiotoxicity is defined, with the most commonly used definition derived from the Cardiac Review and Evaluation Committee (CREC) of trastuzumab-associated cardiotoxicity. The CREC characterizes myocardial toxicity by a symptomatic decrease in left ventricular ejection fraction (LVEF) of at least 5%–55% or an asymptomatic decrease in LVEF of at least 10%–55% [3]. Additional variability in reported cardiotoxicity arises from differing baseline patient characteristics, follow-up times, and a lack of clinical trials reporting predefined cardiac endpoints for chemotherapeutic agents. A comprehensive list of commonly used chemotherapeutic agents, therapeutic indications, and cardiotoxicity rates compiled from relevant studies is presented in Table 1 [4–33].

Table 1. The most frequently used agents in each chemotherapeutic class and their therapeutic indications, along with a range of reported cardiotoxicity rates for each agent

Chemotherapy agent	Cardiotoxicity rate	Therapeutic indications	Notes	References
Anthracyclines				
Doxorubicin (400–700 mg/m^2) Epirubicin (>900 mg/m^2) Idarubicin (>150 mg/m^2) Mitoxantrone (>100 mg/m^2)	3%–48% 0.9%–11.4% 5%–18% 4.1%–14%	Breast cancer Lymphoma/leukemia Lung cancer Sarcoma Ovarian cancer Gastric cancer Liver cancer Thyroid cancer	Cumulative dose-dependent decline in LVEF	[4–13]
Alkylating agents				
Cyclophosphamide Ifosfamide (up to 18 g/m^2)	7%–28% 17%	Lymphoma/leukemia Multiple myeloma Breast cancer Lung cancer Endometrial cancer Sarcoma	Acute onset after initial dose	[14–18]
Antimetabolites				
Clofarabine 5-Fluourouracil[a] Capecitabine[a]	27% <1% <1%	Leukemia Breast cancer Gastric cancer Head and neck tumors Ovarian cancer	[a]High incidence of ischemic symptoms	[8, 19]
Antimicrotubule agents				
Docetaxel Vinorelbine	2.3%–11% 1.2%	Breast cancer Lung cancer Head and neck tumors	Synergistic cardiotoxicity with anthracyclines	[8, 20–22]
Proteasome inhibitors				
Bortezomib	2%	Multiple myeloma Lymphoma		[23]
Monoclonal antibodies				
Trastuzumab Pertuzumab	2%–43.6% 3%–7%	Breast cancer Gastric cancer		[8, 24–26]
Small-molecule TKIs				
Sorafenib Sunitinib Pazopanib Dasatinib Imatinib Lapatinib	6% 2.7%–15% 7%–20% 2%–4% 0.5%–1.7% 1.5%–2.2%	Renal cell cancer Thyroid cancer Breast cancer Leukemia Sarcoma		[8, 27–33]

Doses have been provided for chemotherapeutic agents with demonstrated dose-dependent toxicity.
[a]only for 5-fluourouracil and capecitabine.
Abbreviations: LVEF, left ventricular ejection fraction; TKI, tyrosine kinase inhibitors.

Cancer Therapeutics-Related Cardiotoxicity

Anthracyclines

Anthracyclines are widely used and effective antineoplastic drugs, but cardiotoxicity is a well-established complication of anthracycline cancer therapies. Anthracyclines, such as doxorubicin, are a class of chemotherapeutic agents that inhibit the function of topoisomerase 2B (TOP2B) in cardiomyocytes leading to apoptosis. Progressive cardiotoxicity usually occurs after the completion of treatment with anthracyclines in a dose-dependent manner and may manifest within 1 year (early onset chronic cardiotoxicity) or many years after chemotherapy has been completed (late onset chronic cardiotoxicity) [34].

Monoclonal Antibodies

Trastuzumab has revolutionized the treatment of HER2-positive breast cancer and metastatic gastric cancer. However, clinical trial data on trastuzumab safety has shown a fourfold increase in cardiotoxicity with concurrent trastuzumab and anthracycline treatment, compared with anthracyclines alone [35]. Dysregulation of HER2 signaling suppresses autophagy in cardiomyocytes leading to reactive oxygen species (ROS) accumulation and subsequent cardiotoxicity [36]. Additionally, trastuzumab has been shown to downregulate TOP2B gene expression in primary human cardiomyocytes, which may potentially explain its synergistic cardiotoxicity with anthracyclines [37]. Similarly, newer monoclonal antibodies such as bevacizumab have also been associated with cardiovascular adverse events. Of note, in patients treated with bevacizumab, there is a 4%–35% incidence of hypertension and 2%–4% incidence of heart failure. Bevacizumab inhibits vascular endothelial growth factor (VEGF) and decreases nitric oxide production, leading to hypertension. Consequently, uncontrolled hypertension results in left ventricular hypertrophy and dysfunction. Anti-VEGF effects may also contribute to the

Table 2. This table outlines the antineoplastic mechanism of action for each drug class, focusing on the most commonly used drug in each category, and lists proposed mechanisms of cardiotoxicity for each class

Drug class	Mechanism of antineoplastic action	Mechanism of cardiotoxicity	References
Anthracyclines			
Doxorubicin Epirubicin Daunorubicin	Doxorubicin binds to DNA and TOP2B, causing cell death.	• Free radical accumulation. • Oxidative stress. • TOP2B association with heart failure, targeted by dexrazoxane.	[34, 47–49]
Alkylating agents			
CYC	Attaches an alkyl group to guanine bases in DNA, causing crosslinking and reduced cell proliferation.	• Dose-dependent cardiotoxicity. • Oxidative stress leading to myocardial necrosis and capillary microthrombi formation.	[16, 50]
Antimetabolites			
5-FU	5-FU is a thymidylate synthase inhibitor, which reduces levels of dTMP and consequently inhibits DNA replication.	• 5-FU has the greatest cardiotoxic effect with reported incidences of up to 20%. • Fluoroacetate, a 5-FU metabolite, mediates direct myocardial toxicity and coronary vasospasm.	[51]
Taxanes			
Paclitaxel Docetaxel	Binds to tubulin and prevents depolymerization, leading to microtubule stabilization which limits the progression of the cell cycle.	• Taxane use is associated with bradycardia and ischemia. • Unknown mechanism of cardiotoxicity.	[49]
Monoclonal antibodies			
Trastuzumab Bevacizumab	Targeted therapy against antibodies specific to cancer pathogenesis. Trastuzumab targets the HER2 receptor. Bevacizumab limits angiogenesis via targeted inhibition of VEGFA.	• Trastuzumab: possible inhibition of neuregulin-1 mediated survival and activation of NADPH oxidase via angiotensin II that promotes oxidative stress and downregulation of TOP2B gene expression in cardiomyocytes. • Bevacizumab: VEGF stimulates NO production by upregulating eNOS in endothelial cells. VEGF inhibition causes systemic vasoconstriction and raised blood pressure.	[36–38, 53–55]
TKI			
Imatinib Sunitinib	Overexpression or mutation of tyrosine kinases in malignant cells can increase proliferation and angiogenesis and reduce apoptosis, making it an ideal target in certain cancers.	• Imatinib toxicity is linked to on-target cardiotoxic effects, whereas sunitinib displays off-target effects where unintended kinases are inhibited in cardiomyocytes. • Imatinib (TKI of ABL, KIT, and PDGFRα/β)-ABL inhibition in cardiomyocytes linked to activation of prolonged ER stress response and apoptosis. • Sunitinib—VEGF inhibition leads to hypertension and off-target cardiotoxic side effects of sunitinib possibly from ribosomal S6 kinase inhibition that triggers intrinsic apoptosis by ATP depletion and AMP-activated protein kinase inhibition that stimulates catabolic pathways. • Sunitinib and sorafenib-mediated dysfunction in VEGF–VEGFR signaling impair the angiogenic response necessary to overcome the effects of pressure overload (hypertension-induced) on the heart and prevent the progression to heart failure. • Sorafenib-induced RAF1 antagonism disrupts the ERK cascade, which has cardioprotective effects particularly in response to stress. • KIT receptor inhibition by imatinib, dasatinib, sunitinib, and sorafenib impairs endothelial progenitor cell migration to areas of myocardial infarction where repair is essential to avoid heart remodeling.	[39–46, 56, 57]

(Continues)

Table 2. (*Continued*)

Drug class	Mechanism of antineoplastic action	Mechanism of cardiotoxicity	References
Proteasome inhibitors			
Bortezomib Carfilzomib	The malignant cell may harness the UPP to enhance proliferation and decrease apoptosis. In myeloma cells, PIs activate the UPR causing the accumulation of cytotoxic misfolded or unfolded proteins, eventually leading to apoptosis.	• Cardiotoxic effects linked to UPR in cardiomyocytes, causing apoptosis and are more prevalent in patients with a prior history of chemotherapy or other cardiovascular diseases.	[58–60]

Abbreviations: 5-FU, 5-fluorouracil; ABL, Abelson family of nonreceptor tyrosine kinases; CYC, cyclophosphamide; eNOS, endothelial nitric oxide synthase; ER, endoplasmic reticulum; ERK, extracellular signal-related kinase; KIT, proto-oncogene receptor tyrosine kinase; TKI, tyrosine kinase inhibitors; TOP2B, topoisomerase II-B; PDGFRα/β, platelet-derived growth factor α/β; PI, proteasome inhibitor; UPP, ubiquitin proteasome pathway; UPR, unfolded protein response; VEGFA, vascular endothelial growth factor A.

increased risk of arterial and venous thromboembolism associated with bevacizumab therapy [38].

Tyrosine Kinases Inhibitors

The development of small molecule inhibitors targeting receptor tyrosine kinases that regulate tumor vasculature angiogenesis and cellular proliferation have significantly improved cancer survival outcomes. To inhibit neoplastic cell proliferation, targeted chemotherapeutic agents alter key signaling cascades that are also essential in cardioprotection, especially under stress [39]. However, targeting novel kinases or pathways have been associated with critical cardiovascular side effects due to "on-target" and "off-target" effects [40–60] (Table 2). However, the underlying mechanisms for cardiotoxicity remain unclear. Sunitinib inhibits a wide range of targets including vascular endothelial growth factor receptor (VEGFR), KIT, RET, and platelet-derived growth factor receptor α/β (PDGFRα/β). Hypertension and left ventricular dysfunction are a common adverse effect of sunitinib treatment potentially due to off-target inhibition of ribosomal S6 kinase (RSK) that triggers intrinsic apoptosis by ATP depletion and AMP-activated protein kinase (AMPK) inhibition that stimulates catabolic pathways [28, 41, 42]. Furthermore, imatinib and dasatinib inhibition of the Abelson family (ABL) of nonreceptor tyrosine kinases has been shown to activate the endoplasmic reticulum stress response and induce apoptosis in cardiomyocytes [43]. Other proposed mechanisms for TKI-mediated cardiotoxicity include myocardial contractile dysfunction secondary to disrupted VEGF–VEGFR signaling resulting in an impaired angiogenic response to pressure overload due to hypertension [44], sorafenib-induced RAF1 inhibition which is an essential kinase in the cardioprotective extracellular signal-regulated kinase (ERK) cascade [45] and KIT receptor antagonism that limits endothelial progenitor cell migration to sites of myocardial ischemia [46]. Identifying novel kinases involved in cardiomyocyte function and dysfunction through the "off-target" effects of these multitargeted TKIs can drive future cardiotoxicity and mechanistic studies.

Modeling Anticancer Therapy Mediated Cardiotoxicity In Vitro

To effectively recreate functional cardiac tissues *in vitro* for drug screening, there are three key design elements to be considered—cell source, scaffold design, and biomolecules [61]. In 2006, induced pluripotent stem cells (iPSCs) were established as a potential cell source by the innovative work of Takahashi et al. who used retrovirus-expressed transcription factors to reprogram somatic cells to iPSCs [62]. There are definite advantages of using iPSCs in tissue engineering as they have unlimited expansion capacity, can be derived from several, easily accessible cell types, and can be differentiated into multiple cell lineages. Efficient and chemically directed differentiation protocols have been developed to generate cardiomyocytes from iPSCs [63], which can be further subcategorized into atrial, ventricular, or nodal cells through patch-clamp analysis [64]. Compared with animal models, hiPSC-CMs are more representative of human cardiac physiology in terms of ion channel expression, heart rate, and myofilament composition [65]. Several studies exploring the cardiotoxicity of different chemotherapy agents using stem cell models have been described in the past few years [66–78] (summarized in Table 3).

Anthracyclines

Most of the studies so far have focused on doxorubicin-mediated cardiotoxicity. Burridge et al. [66] identified a differential response to doxorubicin in hiPSC-CMs derived from healthy controls, doxorubicin-treated patients without cardiotoxicity (DOX), and doxorubicin-treated patients with clinical cardiotoxicity (DOXTOX). The DOXTOX cells showed sarcomeric disarray, an increase in arrhythmogenic predisposition, and a decrease in cell viability upon exposure to doxorubicin. The effect of oxidative stress was also explored following doxorubicin administration, with significantly higher levels of induced ROS and a greater decrease in glutathione (GSH) observed in DOXTOX cells. Most interestingly, transcriptomic analysis of doxorubicin treatment identified several differentially regulated genes between DOX and DOXTOX hiPSC-CMs, illustrating the power of this model to unravel the molecular mechanism(s) of inter-individual variation in doxorubicin toxicity. More recently, a panel of hiPSC-CMs derived from 45 individuals was exposed to five different doxorubicin concentrations to generate a comprehensive map of genetic variants [67]. A significant observation from this study was the negative effect of doxorubicin exposure on splicing fidelity, contributing to the high number of genes showing aberrant splicing. Genome editing approaches in hiPSCs have also been tested to elucidate the role of TOP2B in doxorubicin toxicity, a useful tool to further investigate the functional role of other genetic variants. Maillet et al. showed that inactivation of TOP2B via CRISPR/Cas9 resulted in increased cell viability following doxorubicin exposure [68]. Moreover, Gupta et al. described a novel mechanism involving the downregulation of quacking (Qki5), an RNA-binding protein, in doxorubicin-induced cardiotoxicity [69]. Interestingly, Qki5 overexpression attenuated the toxic effect of

Table 3. This table outlines the key findings of each study that uses stem cell models to determine the cardiotoxic effects of different antineoplastic agents

Drug	Key findings	References
Trastuzumab	Detection of trastuzumab-induced cardiotoxicity upon activation of ErbB2/B4 signaling pathway or in coculture with endothelial cells.	[70]
Trastuzumab	Trastuzumab-treated cardiomyocytes showed downregulation of genes involved in small molecule metabolism.	[72]
Pertuzumab Trastuzumab-DM1	Trastuzumab-DM1 displayed a greater decrease in cell viability, compared with pertuzumab alone.	[73]
Trastuzumab Doxorubicin	Inhibition of ErbB signaling with trastuzumab worsened doxorubicin-induced cardiotoxicity.	[71]
Doxorubicin	Comparison of doxorubicin sensitivity in hiPSC-CMs derived from breast cancer patients with induced cardiotoxicity to control hiPSC-CMs mirrored the clinical findings.	[66]
Doxorubicin	RNA-seq analysis on hiPSC-CMs elucidated an *in vitro* transcriptomic response to varying doxorubicin doses that corresponded with cell damage and may be used to predict *in vivo* cardiotoxicity risk.	[67]
Doxorubicin	Doxorubicin demonstrated dose-related hiPSC-CM cell damage, changes in gene expression and electrophysiological abnormalities. CRISPR/Cas9 was used to show the association of TOP2B with doxorubicin-induced cardiotoxicity.	[68]
Doxorubicin	The downregulation of Qki5 in response to doxorubicin increased cardiomyocyte apoptosis.	[69]
Doxorubicin	Vascularized 3D tissue derived from hiPSC-CM demonstrated different cardiotoxic responses in comparison to 2D models.	[75]
Doxorubicin	Doxorubicin tested on hiPSC-CM-derived multiorgan-on-a-chip models revealed marked cardiotoxicity, with increased apoptosis, CK-MB levels, and visible arrhythmia.	[76]
Doxorubicin	48-Hour doxorubicin treatment of a multiorgan-on-a-chip model was evaluated at seven days after treatment, highlighting its effects on drug viability and functionality.	[77]
Tyrosine kinase inhibitors	Cardiac safety indices for 21 TKIs were established using a high-throughput approach. Exogenous insulin and IGF-1 improved hiPSC-CM viability following cotreatment with certain TKIs.	[57]
Sunitinib	Sunitinib-mediated cardiotoxicity on hiPSC-CMs were secondary to multiple kinase inhibition, and not only AMPK and RSK.	[74]
Sunitinib	Increased afterload in 3D microtissues was shown to increase sunitinib-mediated cardiotoxicity *in vitro*, supporting the clinical observation of left ventricular dysfunction following the development of hypertension.	[78]

Abbreviations: AMPK, AMP-activated protein kinase; CK-MB, creatine kinase-MB; CM, cardiomyocyte; hiPSC, human induced pluripotent stem cell; IGF, insulin growth factor; RSK, ribosomal S6 kinase; TKI, tyrosine kinase inhibitors.

doxorubicin through regulation of noncoding circular RNAs derived from *Ttn*, *Fhod3*, and *Strn3* genes, highlighting the potential of harnessing the hiPSC-CMs model to gain mechanistic insights in doxorubicin-induced cardiotoxicity.

Monoclonal Antibodies

A recent study demonstrated that trastuzumab induces cardiotoxicity in hiPSC-CMs that was dependent on the activation of the erythroblastic oncogene B2/B4 (ErbB2/B4) by either neuregulin (NRG-1) or heparin-binding epidermal growth factor, suggesting that trastuzumab is blocking the cardioprotective effects of the ErbB2/4 pathway [70]. In contrast, two other studies showed that trastuzumab-mediated cardiotoxicity on hiPSC-CMs is independent of the ErbB2/B4 pathway activation [71, 72], highlighting the need to develop standardized cell culture conditions to improve the validity of hiPSC-CMs in trastuzumab-toxicity screening. More recently, the potential cardiotoxic effects of pertuzumab and trastuzumab-emtansine (TDM1), a novel antibody–drug conjugate targeting the ErbB2 receptor were tested in the hiPSC-CMs [73]. Of note, clinical trials assessing these agents selected patients without trastuzumab-related cardiotoxicity. Although pertuzumab has been added to the combined treatment regimen for metastatic breast cancer, trastuzumab-DM1 has been approved in metastatic breast cancer resistant to standard therapy as it exhibits more cytotoxic activity than trastuzumab due to the conjugated emtansine (DM1) toxin. Although both pertuzumab and TDM1 showed cardiotoxicity, TDM1 demonstrated a more significant decrease in cell viability as well as marked morphological changes and dysfunction in beating phenotype, emphasizing the utility of hiPSC-CMs as a preclinical model for testing new anticancer drug combinations for cardiotoxicity studies.

Tyrosine Kinase Inhibitors

Patient-specific hiPSC-CMs have already been used to assess the cardiotoxicity of several TKIs, demonstrating the potential of this cell source in high-throughput drug screening for cardio-oncology. Sharma et al. harnessed the ability of hiPSCs to differentiate into multiple lineages to elucidate the cell type-specific cardiotoxic effects of 21 TKIs using a high-throughput approach [57]. An interesting finding from this study was the cardioprotective effect of insulin and insulin growth factor-1 when TKIs inhibited VEGFR and PDGFR. Although VEGFR/PDGFR inhibition leads to cardiotoxicity, this finding suggests that there may be increased sensitivity to pro-survival factors following their inhibition. The hiPSC-CM model has been used in another mechanistic study investigating the role of RSK and AMPK in sunitinib-related cardiotoxicity [74]. In contrast to previous studies on rodent models, specific RSK inhibition did not induce cytotoxicity and pretreatment of hiPSC-CMs with AMPK activators did not alleviate sunitinib-mediated cell death. Although the precise molecular mechanisms of sunitinib-induced cardiotoxicity are

unknown, this study challenged the notion that RSK and AMPK pathways play a causative role in sunitinib-mediated cardiotoxicity, suggesting a key difference between human and rodent cellular models of drug-induced cardiotoxicity.

Refining Cardiac Models for Cardiotoxicity Screening

The hiPSC-CMs most closely resemble human fetal cardiomyocytes in terms of gene expression, ultrastructure, and electrophysiological properties [65]. The lack of T-tubules, the absence of H-zones and M-bands, and poorly developed calcium handling may affect the response of hiPSC-CMs to drugs that affect excitation–contraction coupling [79]. Several methods to promote cardiomyocyte maturation *in vitro* have been proposed including growth factors [80], electrical or mechanical stimulation [81], cell alignment [82], and long-term culture [83], but further refinements are needed to mimic the native heart environment faithfully.

Tissue Engineering

Three-dimensional (3D) engineered heart tissues (EHTs) created using hiPSCs display a more mature phenotype than their two-dimensional (2D) counterparts [84]. Individual approaches with static stretch [85], cyclic stretch [86], and electrical stimulation [85] have also been used to enhance maturation of hiPSC-derived EHTs albeit less effectively than the combinatorial electro-mechanical conditioning [87]. Although cyclic stretch simulates ventricular filling, static stretch recreates embryonic development through progressive lengthening [66]. Together, these mechanical stimulation approaches have been shown to enhance sarcomeric protein structure, cardiomyocyte alignment, calcium cycling, and expression of gap junctions in 3D EHTs derived from hiPSCs [88]. Interestingly, well-aligned cardiac tissue ("biowires") stimulated at high frequency, greater than *in vivo* average heart rates, has also been associated with improved cardiac tissue maturation in terms of size and action potential kinetics [89].

Engineered 3D microtissues from hiPSC-CMs have been used to explore the mechanisms underlying sunitinib-induced cardiotoxicity [90]. Correlating with previous findings, Truitt et al. described a preclinical model that recapitulates cell death and increased caspase 3/7 activation following sunitinib exposure [78]. These findings provide new insight into mechanisms of sunitinib toxicity as they suggest a direct cardiotoxic effect, independent of sunitinib-induced vascular effects. Significantly, the study also observed an increase in caspase activation associated with increased afterload, recreated *in vitro* by altering the stiffness of the pillars to which the 3D tissues are attached. The potential to use 3D tissues for the *in vitro* assessment of increasing afterload on sunitinib cardiotoxicity is promising, with findings supporting the potentiating effect of sunitinib-induced hypertension on left ventricular dysfunction. Clinically, this study implies that early blood pressure control in patients treated with sunitinib may minimize future cardiovascular adverse events.

Doxorubicin has also been tested in both 2D and 3D models derived from hiPSC-CMs. Indeed, a recent study compared the effects of doxorubicin using monolayer-cultured CMs (2D-CM model) and a vascularized 3D EHT iPSC-CM tissues created using nanofilm-based engineering techniques (3D-CM model) [75]. The vascularized 3D-iPSC-CM tissues demonstrated increased resistance to doxorubicin when compared with 2D-iPSC-CM cells. There was no decrease in beating rate when the 3D model was exposed to doxorubicin, compared with a significant decrease in beating rate in the 2D model under the same conditions. Moreover, doxorubicin exhibited a dose-dependent toxic effect on vascularization, suggesting the utility of 3D-CM in evaluating drug-induced vascular toxicity.

Organ-on-a-Chip

Recent developments in microfluidic devices ("organ-on-a-chip" [OOC]) and organoid assembly using hiPSC-CMs provide an opportunity for optimizing chemotherapy-associated cardiotoxicity screening *in vitro*. OOC systems are miniaturized 3D tissue and organ models, which employ a reductionist approach to recapitulate the relevant aspects of organ physiology depending on the eventual application [91]. OOCs offer several advantages as microtissues can be engineered from fewer cells compared with traditional EHTs and are highly reproducible, a critical feature for commercial cardiotoxicity screening. In cardiotoxicity screening for chemotherapy candidates, for example, patient-specific cardiac OOCs must be designed without materials that absorb drugs, include minimal culture media volume to reduce drug dilution, and incorporate microfluidic connections to other OOC models to form a multiorgan chip [91]. An integrative biomimetic platform is essential for drug screening, particularly dual-organ models such as a heart-and-liver-on-a-chip model system, as several chemotherapy agents induce cardiotoxicity after hepatic first-pass metabolism. Examples include doxorubicin that is reduced to the cardiotoxic metabolite, doxorubicinol, by carbonyl reductase-I present in the liver [92] and 5-fluorouracil (5-FU) which is metabolized to cardiotoxic fluoroacetate by α-fluoro-β-alanine, which is a downstream metabolite of 5-FU by dihydropyrimidine dehydrogenase [93].

Cardiac OOC platforms using hiPSCs are being developed for higher throughput drug screening including: muscular thin film based assays to measure contractility [94], 3D bioprinting strategies to fabricate endothelialized-myocardium-on-a-chip [95], pneumatic actuation systems to provide cyclic strain enabling maturation of the 3D constructs along with electrical stimulation [96, 97], and computational modeling of microcirculation to create perfused OOCs with increased functionality [98]. Recently, Zhang et al. reported two multiorgan models, liver-and-heart-on-a-chip and heart-liver-cancer-on-a-chip, with an automated, in situ monitoring system with potentially broad applications in drug toxicity screening [76]. Doxorubicin was used to assess the functionality of this testing model and induced marked cardiotoxicity detected by hiPSC-CM apoptosis, elevated levels of creatine kinase-MB, and arrhythmic beating visualized microscopically [76]. Doxorubicin was also tested in another multi-OOC system where similar effects on cell viability and heart rate were noted with a 65% and 45% decrease, respectively [77]. OOCs offer several advantages as microtissues can be engineered from fewer cells compared with traditional EHTs and are highly reproducible, a critical feature for commercial cardiotoxicity screening.

Cardiac Organoids

Cardiac organoids, on the other hand, are 3D tissue structures arising from the self-assembly of hiPSC-CMs in the presence of

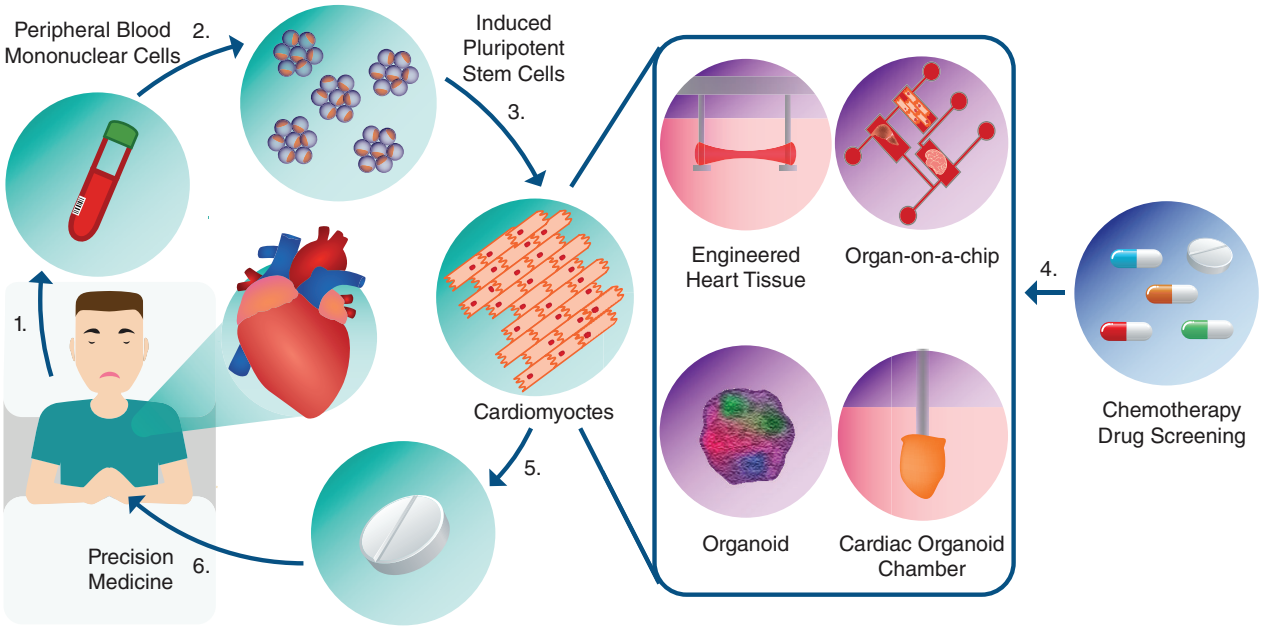

Figure 1. Personalized chemotherapy drug screening to minimize cardiotoxicity. (1) Peripheral blood mononuclear cells (PBMCs) taken from the cancer patient. (2) PBMCs reprogrammed to human induced pluripotent stem cells (hiPSCs). (3) hiPSCs differentiated into cardiomyocytes. (4) Chemotherapy agents screened for toxicity on tissue derived from these cardiomyocytes—engineered heart tissue, organ-on-a-chip, organoid, and cardiac organoid chamber. (5) Single drug with minimal cardiotoxic effects selected from initial drug screen. (6) Tailored therapy for individual patient based on *in vitro* screening.

appropriate factors. The self-organizing properties of stem cells are exploited to create another *in vitro* biological model with potential applications in cardiotoxicity screening. Although stem cells are the key element in organoids, microenvironment design features from biomimetic scaffolds to spatio-temporal control are equally important to coordinate organoid assembly in culture [99]. Spheroid is a term that is sometimes used interchangeably with organoid, but these are distinctly different in that spheroids are 3D aggregates without a stem cell component or tissue-like function [99]. The potential of cardiac organoids in drug toxicity screening has been reinforced by a recent study of environmental toxins on 3D cardiac organoids derived from hiPSC-CMs [100]. When thallium was tested on cardiac organoids, half-maximal inhibitory concentration (IC50) values were similar to lethal patient plasma levels, suggesting the utility of this *in vitro* model in detecting acute toxin effects [99]. This is particularly useful for testing combination chemotherapy regimens due to their magnified risk of acute cardiotoxicity. However, the main challenge of using self-assembled cardiac organoids in drug screening is the lack of an experimentally reproducible model. Hoang et al. have recently described a cell micropatterning approach to overcome this limitation, but the model remains limited to studying early cardiac development and may be useful in cardio-oncology to explore chemotherapy-related fetal cardiac defects [100]. The issue of scalability has also been addressed by high-throughput cardiac organoid screening platforms, such as the Heart-Dyno [101]. Multiple organoids-on-a-chip represent the future of cardiotoxicity screening as they combine the high physiological accuracy of organoids with the ease of automated readouts and perfusability seen in OOC models [102]. Additionally, Li et al. recently reported a 3D human ventricle-like cardiac organoid chamber derived from hiPSC-CMs, with potential to model cardiac pump activity *in vitro* and broader drug screening applications [103].

Conclusion

Cardio-oncology is a constantly evolving clinical discipline, as cardiovascular safety is expected to remain a significant challenge in anticancer therapy secondary to the advent of novel targeted agents. There is increasing interest in identifying the underlying mechanisms of cardiotoxicity induced by both traditional and novel targeted therapies. The advent of hiPSC-CMs and iPSC-CM-derived 3D cultures, such as EHTs, OOC, and organoids, promises to revolutionize preclinical cardiotoxicity drug screening by providing relevant human-based, renewable model systems to explore drug toxicity (Fig. 1). Several studies have demonstrated the utility of the hiPSC-CM-based models to predict the cardiotoxic effects of anticancer therapies, providing novel insights on the underlying molecular mechanisms of cardiotoxicity. Although there is a need for improved protocols to address the relative immaturity of hiPSC-CMs, the patient-specific hiPSC-CM technology can serve as a platform for personalized medicine. Nevertheless, for effective translation into cardio-oncology clinical practice, results from existing *in vivo* and *in silico* models must be combined with high fidelity *in vitro* models to better predict chemotherapy-induced cardiotoxicity and maximize patient safety.

Disclosure of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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