Advancing cardiac safety and drug discovery screening using human stem cell-derived cardiomyocytes

Korte, T. de

Modelling the physiology and pathophysiology of the human heart has, until recently, been a major challenge in biomedicine. This has resulted in poorly understood and unexpected cardiotoxicities and untreatable cardiac diseases, contributing significantly to global morbidity and mortality. Human-induced pluripotent stem cell-derived cardiomyocytes (hiPSCs-CMs) are now presenting a promising alternative to widely used mouse models as preclinical models since they closely mirror human physiological conditions, offering insights into cardiac diseases and drug responses. However, their widespread adoption is hindered by their variability in response, immaturity, scalability, and associated costs. This thesis delves into the expansive utilization of both 2D and 3D hiPSC-CM models for assessing cardiac safety and efficacy through a combination of *in vitro* and in silico methodologies (Figure 1). Various technological innovations are described that led to hiPSC-CM models that are more scalable, reproducible and in which the hiPSC-CMs are more mature. These advancements have elevated the "technology-readiness-level" of the model, making it readily applicable for modelling certain diseases and for high-throughput screening, particularly suitable for use within the pharmaceutical industry. With continued advancements, such as those described in this thesis, hiPSC-CMs could transform drug discovery for cardiovascular disease, safety testing, and personalized medicine.

1

General introduction and thesis outline

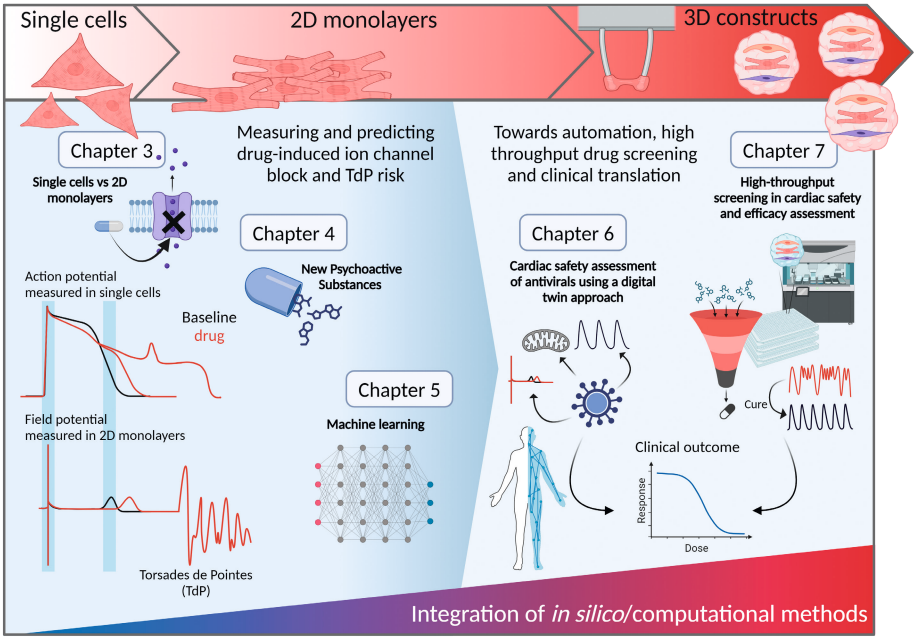

Figure 1. Graphical abstract of the thesis outline.

Cardiovascular disease and cardiotoxicity

Cardiovascular diseases (CVDs) collectively encompass ischemic heart disease, stroke, heart failure, peripheral arterial disease, and various other cardiac and vascular conditions; they remain leading causes of global mortality and significantly diminish quality of life for many individuals.[1–3] According to the World Health Organization (WHO), CVDs accounted for an estimated 17.9 million deaths in 2019, constituting 32% of all global mortality.[4] Additionally, the projected global economic burden of CVDs is staggering, expected to reach €950 million by 2030.[5] Among the primary causes of cardiovascular death are high blood pressure, dietary risks, elevated LDL cholesterol, air pollution, and tobacco use.[3] However, the impact of genetic factors cannot be overlooked. The majority of CVDs and their risk factors have polygenic origins, involving a complex interplay between environmental factors, lifestyle choices, and multiple genetic variants.[6] Furthermore, monogenetic conditions can lead to severe premature CVDs and early mortality if left untreated.[6] These inherited CVDs cover a range of conditions, from more common ailments like hypertrophic cardiomyopathy (HCM) and familial hypercholesterolemia (FH) to rarer inherited arrhythmia syndromes such as long QT syndrome (LQTS), Brugada syndrome (BrS), and catecholaminergic polymorphic ventricular tachycardia (CPVT).[7] CPVT will be further discussed in detail in this thesis.

Beyond inherited susceptibility to CVD, cardiovascular toxicity resulting from therapeutic drug usage accounts for a significant incidence and severity of adverse drug reactions during late-stage clinical development.[8,9] Concerns regarding cardiac safety are among the top reasons for drug attrition from clinical trials and the market.[10] Currently, 90% of the drug candidates that pass preclinical phases fail during clinical trials and approval processes, with lack of clinical efficacy and unmanageable toxicity being primary causes.[11] In particular, many novel drug candidates targeting cancer cells are withdrawn due to adverse cardiotoxic effects, even after extensive testing in animal models,[12] indicating the limited predictivity of current preclinical models. In fact, it has been suggested that up to 70% of cardiotoxicities observed in clinical trials could have been predicted with improved preclinical screening methods.[13] While preclinical animal models have contributed significantly to our understanding of disease phenotypes, ethical concerns on instrumental animal use and physiological disparities between animal and human systems limit their utility in studying CVDs and cardiotoxicities. Most notably, there are significant differences between cardiomyocytes from small animal models and humans, including heart rates, energetics, calcium cycling, myofilament composition, ion channel expression, and cellular electrophysiology.[14] For example, the resting heart rate of a mouse is ~500–700 beats per minute, roughly 10 times faster than in a resting human. In addition, the mouse modulates heart rate minimally; even during strenuous exercise as it only increases by 50% at most, whereas in humans the heart rate can increase by up to 300%.[15] Consequently, drug-, disease-, or stress-induced effects on heart rate and kinetics are challenging to study in mice.

To address these limitations, cellular models may offer an alternative, and at the same time overcoming issues related to throughput, costs, and ethical concerns associated with *in vivo* models. However, existing *in vitro* models, often employ simplified (non-)human cell lines over- or ectopically expressing ion channels and lacking the complex electrophysiological and mechanistic interactions that determine the properties of human myocardium. While primary human cardiomyocytes (CMs) hold promise for more accurate modeling, their scarcity, difficulty in harvesting, high cost, and limited proliferative capacity pose significant challenges for widespread use in pharmaceutical research.[16] Developing reliable, sensitive, and clinically relevant human *in vitro* CM models are imperative for advancing CVD modeling and drug efficacy and safety assessment.

The discovery and potential of human-induced pluripotent stem cells

An emerging class of *in vitro* models recapitulating both the complexity of patient-specific physiology and the cardiac electrophysiology are hiPSC-CMs. Pluripotent stem cells (PSCs) can divide indefinitely and are capable of differentiating to any cell type of the human body. The first human PSCs were isolated as embryonic stem cells (hESC) in 1998 from the late blastocyst stage embryos donated for research purposes after becoming surplus to requirements

for treating infertility. Since this raised ethical concerns because the embryo is destroyed by generating hESCs, many efforts were made to identify other sources for hPSCs. This eventually led to the discovery of induced PSCs (iPSCs) in mice by Takahashi and Yamanaka in 2006[17] and one year later, in humans.[18] This groundbreaking research built upon Gurdon's work in 1958, where he demonstrated the reversible nature of cell specialization by showing that an adult cell nucleus transferred to unfertilized egg could revert to a pluripotent state by reprogramming through cloning.[19] More than four decades later, Takahashi and Yamanaka successfully reprogrammed intact mature human cells into hiPSCs by expressing of a specific set of transcription factors (OCT4, SOX2, c-MYC, and Klf4, referred to as OSKM).[18] In 2012, these breakthroughs led to the Nobel Prize in Physiology or Medicine for Gurdon and Yamanaka for their joint discovery that mature cells possess the remarkable ability to be reprogrammed to pluripotency. Today, hiPSCs can be created with relative ease from a wide variety of somatic cell sources, including skin fibroblasts, mononuclear blood cells, and cells in urine.[20,21] They have emerged as a versatile tool in biomedical and pharmaceutical research, demonstrating the capacity to differentiate into various cell types found in the human body. These include hepatocytes, neurons, blood cells, cardiomyocytes, and more, facilitating the study of human (patho)physiology using both donor-derived healthy- and diseased cell lines.

The differentiation of hiPSCs towards cardiomyocytes can now be achieved using any one of a number of high-efficiency protocols, whereby more than 95% of the differentiated population can be made up of cardiomyocytes from their initial hiPSC population. These hiPSC-CMs exhibit many functional and structural features of *bona fide* cardiomyocytes. Additional possibilities have been created by improvements in genetic engineering of hiPSCs. For example, using efficient gene-editing approaches, such as CRISPR/Cas9[22], it is now possible to create hiPSC-CMs that carry targeted gene knockouts, knock-ins, or polymorphic substitutions, allowing researchers to explore disease-specific phenotypes within hiPSC-CMs possessing identical genetic backgrounds. Whether technologies using hiPSC-CMs could indeed complement existing assays to improve cardiac safety assessment and drug efficacy screening while reducing socioeconomic costs and assisting with regulatory guidelines is extensively reviewed in **chapter 2**. This chapter elucidates both the potential and limitations associated with the use of hiPSC-CMs, including its maturity versus complexity, consistency, quality, and cost.

Characteristics of human-induced pluripotent stem cell-derived cardiomyocytes

Human iPSC-CMs provide an unlimited and renewable source of cardiomyocytes, exhibiting the major cardiac structural proteins[23], ion channels, calcium (Ca^{2+}) cycling components[24], and adrenergic receptors. As such, hiPSC-CMs can be subjected to detailed analysis of their molecular, pharmacological, electrophysiological and contractile properties, yielding valuable mechanistic insight relevant to basic cardiac research and drug development. Cardiomyocyte

electrophysiology revolves around the action potential (AP), driven by ion movement involving sodium (Na^+), potassium (K^+), and calcium (Ca^{2+}) via channels and transporters. Key ion channels and transporters underlying the ventricular cardiomyocyte AP are encoded by the genes KCND2/3, KCNIP2, and DPP6/10 for producing the I_{to} current, and the genes SCN5A, CACNA1C, KCNH2 (hERG), KCNQ1, KCNJ2/12/4/14 and SLC8A1-A3 for producing the I_{Na}, I_{CaL}, I_{kr}, I_{ks}, I_{k1}, and I_{NCX} currents, respectively.[25] During depolarization of the AP, I_{CaL} and I_{NCX} increase the free cytosolic Ca^{2+}, triggering Ca^{2+}-induced Ca^{2+} release (CICR) from the sarcoplasmic reticulum (SR) via ryanodine receptors (RyR) and raising intracellular Ca^{2+} levels. Ca^{2+} subsequently binds to the sarcomeres and allows the movement of myofilaments, which causes contraction. The cell membrane is then repolarized by several repolarizing potassium currents and Ca^{2+} is removed from the cytosol, either by re-sequestering it in the SR via the sarco-endoplasmic reticulum ATP-ase (SERCA), or extruding it into the extracellular space through the Na^+-Ca^{2+} exchanger (NCX) or Ca^{2+} ATPase pump, leading to relaxation. Functional changes in any of these currents could have important consequences for the electrophysiological properties of the heart (i.e., action potential shape, refractory periods, and cardiac rhythms). An understanding of hiPSC-CM AP properties is therefore critical for an accurate assessment of the cardiac electrical and contractile function as well as the functional maturity of the cells. There are various techniques available to study the electrophysiological properties of hiPSC-CMs, including patch clamp, multi-electrode array (MEA), and fluorescence dye-based assessment of the membrane potential. MEA allows non-invasive, kinetic recording of extracellular field potentials (FPs) of electrically coupled cardiomyocyte monolayers and correlate with APs typically measured via patch-clamp. Each technique has distinct advantages and limitations, detailed across various chapters of this thesis. In **chapter 3**, we adopted a multilevel approach, utilizing both patch clamp and MEA technologies to explore electrophysiology, focusing on repolarization instability—a novel and critical parameter in proarrhythmic risk evaluation. **Chapter 4** further underscores the importance of MEA recordings in hiPSC-CMs, particularly for rapid screening applications. This is exemplified by the evaluation of numerous potentially cardiotoxic new psychoactive substances (NPS) flooding today's drug markets. This chapter demonstrates how hiPSC-CMs efficiently detect direct cardiotoxic effects of NPS, offering swift and reliable insights that could otherwise be challenging or unfeasible with conventional pre-clinical methods.

The role of human-induced pluripotent stem cell-derived cardiomyocytes in rewriting regulatory guidelines on cardiac safety assessment

Soon after the first derivation of hiPSC-CMs, their value in drug evaluation was identified, particularly for ion channel modulators affecting cardiac electrophysiology. Modulating ion channels and transporters can disrupt the AP, leading to arrhythmias, like Torsades de Pointes ("twisting of points", TdP), which is a life-threatening polymorphic ventricular tachycardia. TdP is marked by AP duration (APD) prolongation in ventricular cardiomyocytes and, hence,

prolongation of the QT interval on the electrocardiogram (ECG). François Dessertenne initially described TdP in 1966. Based on clinical cases from more than a century ago[26], he found that patients treated with quinidine showed prolonged QT intervals that were associated with "rhythms indicating intoxication of the heart muscle". TdP, once thought rare and linked mainly to antiarrhythmic drugs, saw a surge in cases in the late 1980s and early 1990s. It became known that noncardiac drugs taken by millions of patients, such as the antihistamine terfenadine, could also cause TdP. Consequently, drugs such as astemizole, cisapride, sertindole, and droperidol were withdrawn from the U.S. and European markets.[27,28]

To offer guidance in detecting drug-induced TdP, regulatory agencies introduced the ICH S7B and ICH E14 guidelines in 2005, focusing on *in vitro* hERG channel assays and *in vivo* QT measurements to predict TdP risk. Although effective, the hERG assays often employ simplified cell models (e.g., using CHO or HEK cell lines) overexpressing I_{Kr}, lacking the ion channel complexity of cardiomyocytes. This approach has led to unnecessary drug attrition and high costs.[29] For instance, verapamil, initially flagged as potential harmful based on hERG assays, is considered safe due to its dual blockage of hERG and L-type Ca^{2+} channels, detectable using hiPSC-CMs.[30] Reliance on simplified models and overemphasis on hERG blockage and QT prolongation may have hindered the market entry of beneficial drugs. The demand for integrated human models like hiPSC-CMs, capable of accurately assessing drug effects on multiple cardiac ion channels, has prompted a paradigm shift in cardiac safety assessment, predominantly driven by the Comprehensive *In Vitro* Proarrhythmia Assay (CiPA) initiative.[31–33] CiPA, initiated by Cardiac Safety Research Consortium (CSRC), Health and Environmental Sciences Institute (HESI), and the FDA in July 2013, aims to improve the specificity of proarrhythmic risk assessment. Overseen by a steering team comprising international regulators, industry, academics, and nonprofits, CiPA operated through four main working groups: Ion Channel, In Silico, Stem Cell Derived Myocytes, and Clinical ECG. The preclinical groups focussed on mechanistic assessments of drug effects on various cardiac ion channel types, employing ion channel screening, *in silico* modelling, and testing on hiPSC-CMs. Within these efforts, the first international multisite study utilizing hiPSC-CMs in drug evaluation for proarrhythmia was initiated.[33] This study demonstrated the utility of hiPSC-CMs in detecting drug-induced proarrhythmic effects with a predictivity of 82-87% for 28 drugs across low, intermediate, and high TdP risk categories. Building upon this work, **chapter 5** describes an integrated approach of hiPSC-CM *in vitro* studies with *in silico* modelling to enhance machine learning (ML) approaches for predicting TdP risk and ion channel blockade of the "CiPA drugs". As larger and more complex datasets emerge from novel *in vitro* studies, ML algorithms are anticipated to be integrated more frequently. This advancement not only streamlines the assessment of drug-induced ion channel blockade and proarrhythmic behaviour, but could also contribute to the discovery of novel biomarkers. According to the novel S7B/E14 Q&A document that was published as a result of the CiPA studies in 2022, hiPSC-CMs are now recommended as appropriate for proarrhythmia risk assessment, particularly in follow-up investigation when nonclinical core assays are not "double-negative" (i.e. negative *in vitro* hERG and *in vivo* heart-rate corrected QT).[34] Such an

integrated risk assessment with double-negative nonclinical findings will hopefully alleviate the pharmaceutical industry's burden of unnecessary clinical QTc studies[29], streamline drug development, and contribute to the reduction, refinement and replacement of animal research (the 3Rs). However, considerable challenges remain regarding the use of hiPSC-CMs. For example, hiPSC-CMs have characteristics more akin to immature cardiomyocytes than fully differentiated adult cardiomyocytes, as evidenced by disorganized sarcomeres, high resting membrane potential, and lower contraction force.[35,36] The lack of a fully mature phenotype, however, may be irrelevant if the standard criteria for model validation (no false positives or negatives) are met.

Broad potential of using hiPSC-CMs for assessing cardiac safety and drug efficacy

The CiPA initiative marks a significant shift in proarrhythmic risk assessment, yet its focus primarily on acute effects of drugs with a hERG-related mode of action leaves room for further exploration. Beyond hERG, examining potential delayed effects on ion channels (e.g., hERG trafficking) presents another opportunity. Moreover, aside from influencing safety decisions regarding drug-induced proarrhythmia, hiPSC-CMs hold immense potential in assessing non-proarrhythmic cardiotoxicity (e.g., contractile, structural, and metabolic toxicities), where current non-clinical approaches fall short in early detection before clinical trials. Assessing cardiotoxicity at multiple levels will allow more reliable risk assessment and lead to more informed regulatory decisions early in drug development. **Chapter 6** presents an integrated, time-efficient *in vitro/in silico* approach for comprehensive cardiotoxicity assessment of antivirals, crucial for accurate translation to clinical settings. It emphasizes the significance of utilizing hiPSC-CMs for both acute and long-term cardiac safety testing of antivirals, especially crucial during viral outbreaks like the COVID-19 pandemic. Beyond evaluating electrophysiology, metabolic activity, and viability, this chapter explores drug effects on the structure and contractility of hiPSC-CMs modelled in 3D. Developing hiPSC-CM models in 3D holds promise for better replication of contractile properties of adult ventricular myocytes and achieving an overall more mature phenotype.[37,38] Additional strategies to enhance the maturity of cardiomyocytes in *in vitro* models include incorporating physiological substrates, applying electrical or mechanical stimulation[39], prolonging culture duration, adding supplements such as hormones[40], and co-culturing with endothelial cells and/or fibroblasts[37]. Despite significant progress in creating more mature and advanced cardiac models to enhance physiological relevance and reduce reliance on animal models, several challenges persist. These challenges are related to reproducibility, scalability, compatibility with established assays, and the requirement for specialized expertise, substantial cell numbers, or specific equipment. Consequently, these obstacles impede routine implementation in high throughput screening (HTS) processes. In **chapter 7**, we address these critical challenges and underscore the need and process to make hiPSC-CMs ready for adoption in the pharmaceutical industry. Expanding on prior research demonstrating that a combination of hiPSC-CMs, hiPSC-cardiac endothelial

cells, and hiPSC-cardiac fibroblasts within 3D cardiac microtissues (cMTs) enhances maturation and reveals the non-cardiomyocyte contributions to heart disease,[37] this chapter advances the field by introducing a streamlined methodology integrating robotics and automation. This innovative approach elevates the quality and utility of cMTs to exceptional levels. The optimized cMTs exhibit enhanced reproducibility and improved predictivity when exposed to inotropic compounds. In addition, they enable the robust manifestation of the cardiac disease phenotype, CPVT, in high-throughput settings—an achievement thus far elusive with similar stem cell models.

Chapter 8 concludes this thesis by providing further light on the advances and integration of novel technologies complementing hiPSC-CM models in their use for drug discovery and development. By embracing standardization, automation, and robotics, researchers can significantly bolster the efficiency and reliability of hiPSC-CM models for drug screening, thereby reducing costs, accelerating development timelines, and advancing the translation into tangible clinical applications.

References

1. Lindstrom, M. et al. Global Burden of Cardiovascular Diseases and Risks Collaboration, 1990-2021. J Am Coll Cardiol 80, 2372–2425 (2022).
2. Mensah, G. A., Roth, G. A. & Fuster, V. The Global Burden of Cardiovascular Diseases and Risk Factors. J Am Coll Cardiol 74, 2529–2532 (2019).
3. Vaduganathan, M., Mensah, G. A., Turco, J. V., Fuster, V. & Roth, G. A. The Global Burden of Cardiovascular Diseases and Risk. J Am Coll Cardiol 80, 2361–2371 (2022).
4. World Health Organization. Cardiovascular diseases (CVDs). https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds) (2021).
5. Timmis, A. et al. European Society of Cardiology: Cardiovascular Disease Statistics 2019. Eur Heart J 41, 12–85 (2020).
6. Vrablik, M., Dlouha, D., Todorovova, V., Stefler, D. & Hubacek, J. A. Genetics of Cardiovascular Disease: How Far Are We from Personalized CVD Risk Prediction and Management? Int J Mol Sci 22, 4182 (2021).
7. Ingles, J., Macciocca, I., Morales, A. & Thomson, K. Genetic Testing in Inherited Heart Diseases. Heart Lung Circ 29, 505–511 (2020).
8. Savoji, H. et al. Cardiovascular disease models: A game changing paradigm in drug discovery and screening. Biomaterials 198, 3–26 (2019).
9. Ferri, N. et al. Drug attrition during pre-clinical and clinical development: Understanding and managing drug-induced cardiotoxicity. Pharmacol Ther 138, 470–484 (2013).
10. Mamoshina, P., Rodriguez, B. & Bueno-Orovio, A. Toward a broader view of mechanisms of drug cardiotoxicity. Cell Rep Med 2, 100216 (2021).
11. Sun, D., Gao, W., Hu, H. & Zhou, S. Why 90% of clinical drug development fails and how to improve it? Acta Pharm Sin B 12, 3049–3062 (2022).
12. Kumar, M., Thangavel, C., Becker, R. C. & Sadayappan, S. Monoclonal Antibody-Based Immunotherapy and Its Role in the Development of Cardiac Toxicity. Cancers (Basel) 13, 86 (2020).
13. Eder, A., Vollert, I., Hansen, A. & Eschenhagen, T. Human engineered heart tissue as a model system for drug testing. Adv Drug Deliv Rev 96, 214–224 (2016).
14. Karakikes, I., Ameen, M., Termglinchan, V. & Wu, J. C. Human Induced Pluripotent Stem Cell–Derived Cardiomyocytes. Circ Res 117, 80–88 (2015).
15. Janssen, P. M. L., Biesiadecki, B. J., Ziolo, M. T. & Davis, J. P. The need for speed: Mice, men, and myocardial kinetic reserve. Circ Res 119, 418–421 (2016).
16. Khan, J. M., Lyon, A. R. & Harding, S. E. The case for induced pluripotent stem cell-derived cardiomyocytes in pharmacological screening. Br J Pharmacol 169, 304–317 (2013).
17. Takahashi, K. & Yamanaka, S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Cell 126, 663–676 (2006).
18. Takahashi, K. et al. Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. Cell 131, 861–872 (2007).
19. GURDON, J. B., ELSDALE, T. R. & FISCHBERG, M. Sexually Mature Individuals of Xenopus laevis from the Transplantation of Single Somatic Nuclei. Nature 182, 64–65 (1958).
20. Raab, S., Klingenstein, M., Liebau, S. & Linta, L. A Comparative View on Human Somatic Cell Sources for iPSC Generation. Stem Cells Int 2014, 1–12 (2014).
21. Xue, Y. et al. Generating a Non-Integrating Human Induced Pluripotent Stem Cell Bank from Urine-Derived Cells. PLoS One 8, (2013).
22. De Masi, C., Spitalieri, P., Murdocca, M., Novelli, G. & Sangiuolo, F. Application of CRISPR/Cas9 to human-induced pluripotent stem cells: from gene editing to drug discovery. Hum Genomics 14, 25 (2020).

23. Kujala, K. et al. Cell Model of Catecholaminergic Polymorphic Ventricular Tachycardia Reveals Early and Delayed Afterdepolarizations. PLoS One 7, (2012).
24. Itzhaki, I. et al. Calcium handling in human induced pluripotent stem cell derived cardiomyocytes. PLoS One 6, e18037 (2011).
25. Varró, A. et al. Cardiac transmembrane ion channels and action potentials: cellular physiology and arrhythmogenic behavior. Physiol Rev 101, 1083–1176 (2021).
26. Archibald, E. & Clark-Kennedy. Quinidine in the Treatment of Auricular Fibrillation. QJM: An International Journal of Medicine os-16, 204–235 (1923).
27. Zhang, Z., Zhou, H., Yang, Y., Liu, L. & Liu, X. Assessment of Quinidine-Induced Torsades de Pointes Risks Using a Whole-Body Physiologically Based Pharmacokinetic Model Linked to Cardiac Ionic Current Inhibition. Clin Pharmacol Ther 115, 616–626 (2024).
28. Stockbridge, N., Morganroth, J., Shah, R. R. & Garnett, C. Dealing with Global Safety Issues. Drug Saf 36, 167–182 (2013).
29. Vargas, H. M. et al. Time for a Fully Integrated Nonclinical–Clinical Risk Assessment to Streamline QT Prolongation Liability Determinations: A Pharma Industry Perspective. Clin Pharmacol Ther 109, 310–318 (2021).
30. Navarrete, E. G. et al. Screening drug-induced arrhythmia events using human induced pluripotent stem cell-derived cardiomyocytes and low-impedance microelectrode arrays. Circulation 128, (2013).
31. Fermini, B. et al. A New Perspective in the Field of Cardiac Safety Testing through the Comprehensive *In Vitro* Proarrhythmia Assay Paradigm. SLAS Discovery 21, 1–11 (2016).
32. Sager, P. T., Gintant, G., Turner, J. R., Pettit, S. & Stockbridge, N. Rechanneling the cardiac proarrhythmia safety paradigm: A meeting report from the Cardiac Safety Research Consortium. Am Heart J 167, 292–300 (2014).
33. Blinova, K. et al. International Multisite Study of Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes for Drug Proarrhythmic Potential Assessment. Cell Rep 24, 3582–3592 (2018).
34. European Medicines Agency. ICH Guideline E14/S7B: Clinical and Nonclinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential-Questions and Answers. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-e14/s7b-clinical-nonclinical-evaluation-qt/qtc-interval-prolongation-proarrhythmic-potential-questions-answers-step-5_en.pdf (2022).
35. Yang, X., Pabon, L. & Murry, C. E. Engineering adolescence: Maturation of human pluripotent stem cell-derived cardiomyocytes. Circ Res 114, 511–523 (2014).
36. Pugsley, M. K. et al. Safety pharmacology 2023 and implementation of the ICH E14/S7B Q&A guidance document. J Pharmacol Toxicol Methods 123, (2023).
37. Giacomelli, E. et al. Human-iPSC-Derived Cardiac Stromal Cells Enhance Maturation in 3D Cardiac Microtissues and Reveal Non-cardiomyocyte Contributions to Heart Disease. Cell Stem Cell 26, 862-879.e11 (2020).
38. Mannhardt, I. et al. Human Engineered Heart Tissue: Analysis of Contractile Force. Stem Cell Reports 7, 29–42 (2016).
39. Ruan, J. L. et al. Mechanical Stress Conditioning and Electrical Stimulation Promote Contractility and Force Maturation of Induced Pluripotent Stem Cell-Derived Human Cardiac Tissue. Circulation 134, 1557–1567 (2016).
40. Yang, X. et al. Tri-iodo-l-thyronine promotes the maturation of human cardiomyocytes-derived from induced pluripotent stem cells. J Mol Cell Cardiol 72, 296–304 (2014).