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REVIEW ARTICLE

Promising tools for future drug discovery and development in antiarrhythmic therapy

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ABSTRACT

Arrhythmia refers to irregularities in the rate and rhythm of the heart, with symptoms spanning from mild palpitations to life-threatening arrhythmias and sudden cardiac death. The complex molecular nature of arrhythmias complicates the selection of appropriate treatment. Current therapies involve the use of antiarrhythmic drugs (class I–IV) with limited efficacy and dangerous side effects and implantable pacemakers and cardioverter-defibrillators with hardware-related complications and inappropriate shocks. The number of novel antiarrhythmic drugs in the development pipeline has decreased substantially during the last decade and underscores uncertainties regarding future developments in this field. Consequently, arrhythmia treatment poses significant challenges, prompting the need for alternative approaches. Remarkably, innovative drug discovery and development technologies show promise in helping advance antiarrhythmic therapies. In this article, we review unique characteristics and the transformative potential of emerging technologies that offer unprecedented opportunities for transitioning from traditional antiarrhythmics to next-generation therapies. We assess stem cell technology, emphasizing the utility of innovative cell profiling using multiomics, high-throughput screening, and advanced computational modeling in developing treatments tailored precisely to individual genetic and physiological profiles. We offer insights into gene therapy, peptide, and peptibody approaches for drug delivery. We finally discuss potential strengths and weaknesses of such techniques in reducing adverse effects and enhancing overall treatment outcomes, leading to more effective, specific, and safer therapies. Altogether, this comprehensive overview introduces innovative avenues for personalized rhythm

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therapy, with particular emphasis on drug discovery, aiming to advance the arrhythmia treatment landscape and the prevention of sudden cardiac death.

Significance Statement: Arrhythmias and sudden cardiac death account for 15%–20% of deaths worldwide. However, current antiarrhythmic therapies are ineffective and have dangerous side effects. Here, we review the field of arrhythmia treatment underscoring the slow progress in advancing the cardiac rhythm therapy pipeline and the uncertainties regarding evolution of this field. We provide information on how emerging technological and experimental tools can help accelerate progress and address the limitations of antiarrhythmic drug discovery.

I. Introduction

Cardiac arrhythmias are abnormal heart rhythms that can lead to serious health problems, including heart failure and sudden cardiac death. Current antiarrhythmic treatments are empirical and subject to clinical judgment. They remain a weakness in contemporary cardiovascular medicine (Schwartz et al, 2020; Kingma et al, 2023). A broad pharmacological arsenal designed to modulate cardiac electrical activity exists aiming to restore the rhythm (Kingma et al, 2023). However, the complex nature of arrhythmias, coupled with limited effectiveness plus side effects associated with current therapeutic approaches, underscores the need for innovative strategies to propel the field forward. Fortunately, several emerging technologies are showing promise in the field of drug discovery and the development of antiarrhythmic therapies. Such technologies offer unprecedented opportunities to revolutionize drug discovery and development, marking the onset of a paradigm shift in the search for novel antiarrhythmic therapies. Transition from traditional antiarrhythmics to next-generation therapies may boost precision medicine, with treatments tailored to individual genetic, physiological, and environmental profiles. In this review, we explore for potential antiarrhythmic drug discovery and development tools by first delving briefly into the current landscape of antiarrhythmic therapy, critically evaluating the strengths and limitations of existing pharmaceutical agents (Valderrábano, 2022; Saljic et al, 2023). Subsequent sections describe the transformative potential of stem cell technology, multiomics, advanced computational modeling, and high-throughput screening (HTS), all of which are gaining attention in drug discovery and development. We also look at the future potential of gene and peptide-based therapies in treating cardiac arrhythmias. Together with an ever-increasing understanding of the molecular mechanisms underlying cardiac arrhythmias, these technologies support an optimistic outlook toward improved pharmacological treatment opportunities for patients with cardiac arrhythmias. These auspicious tools also offer a better understanding of the intricacies of cardiac electrophysiology. They should help researchers develop new, more specific, safe, and effective therapies that are also tailored to the unique characteristics of each patient, promoting personalized medicine (Piccini et al, 2022). Therefore, this review aimed to navigate the trajectories toward future alternatives in antiarrhythmic therapies, highlighting promises and challenges associated with them.

II. The traditional landscape of antiarrhythmic drug therapy

Traditional antiarrhythmic drugs (AADs), categorized by their electrophysiological effects, are currently what is best for controlling the electrical activity of the heart and managing rhythm disturbances. Selecting the appropriate antiarrhythmic therapy depends on the specific type of arrhythmia, its underlying cause, and individual patient characteristics (Al-Khatib et al, 2018; Michowitz et al, 2021). Sodium channel blockers, or class I AADs (quinidine, procainamide, disopyramide, lidocaine, flecainide, and propafenone), inhibit sodium channels during depolarization, slowing the rate of rise of the action potential (AP) and thus reducing cell excitability and conduction velocity (Lei et al, 2018). Class Ic AADs agents, such as flecainide and propafenone, also exert their antiarrhythmic effects by targeting the ryanodine receptor (RyR) 2, a critical calcium release channel in the heart (Watanabe et al, 2009; Hilliard et al, 2010; Kryshtal et al, 2021; Salvage et al, 2022). This action helps stabilize calcium handling and reduces the risk of arrhythmogenic events, making these drugs particularly effective in treating certain types of arrhythmias where calcium dysregulation plays a role (Kryshtal et al, 2021; Li et al, 2022; Bergeman et al, 2023). β-Adrenergic receptor blockers, or class II AADs such as propranolol, metoprolol, and atenolol, reduce sympathetic stimulation and decrease heart rate (HR) and contractility (Wołowiec et al, 2022). Class III AADs are potassium channel blockers (eg, amiodarone, sotalol, and dofetilide) that prolong the repolarization phase of the AP (Roden, 2016; Pannone et al, 2021). Similar to class II, calcium channel blockers (class IV AADs), including verapamil and diltiazem, decrease the HR and contractility by inhibiting the calcium influx during AP depolarization (Koldenhof et al, 2023; Meyer et al, 2023). There are other drugs not categorized within these 4 classes that exhibit antiarrhythmic actions. For example, digoxin, traditionally used in the most common clinical arrhythmia, atrial fibrillation (AF), increases the force of myocardial contraction and impairs conduction through the atrioventricular node (AVN) (Ziff and Kotecha, 2016). Adenosine, used to treat supraventricular tachycardias, also slows conduction through the AVN and interrupts re-entry across accessory AVN pathways (Ziff and Kotecha, 2016; Gupta et al, 2021). Ranolazine is used in certain cases of angina and has also shown antiarrhythmic efficacy by inhibiting the late sodium current (I_{Na}), reducing calcium overload (Frommeyer et al, 2016; Shenasa et al, 2016; Rouhana et al, 2021). Vernakalant, a relatively novel therapy for AF, also shows antiarrhythmics effects blocking multiple ion channels (Frommeyer et al, 2016, 2017; Hall and Mitchell, 2019). While these drugs have undoubtedly improved patient outcomes, their use must be carefully selected and monitored owing to potential proarrhythmia (ie, inducing new arrhythmias), limited efficacy, and adverse side effects. When pharmacologic therapy is not sufficient, nonpharmacological interventions such as catheter ablation and implantable devices are commonly used in the management of certain arrhythmias. Implantable cardioverter-defibrillators are implanted to detect and treat life-threatening ventricular arrhythmias by delivering an electric shock to restore normal rhythm. The use of pacemakers helps to coordinate contraction between the heart chambers (Gopinathannair et al, 2019; Elsokkari and Sapp,

2021; Arenal et al, 2022). However, it is extremely important to note that patients treated with AADs or nonpharmacological interventions are not exempt from sudden cardiac death (SCD) risk (Priori et al, 2015; Richards et al, 2015; Mazzanti et al, 2020).

Importantly, the persistent use of class I and class II antiarrhythmic agents is associated with a significant risk of proarrhythmic effects and increased mortality, particularly in patients with underlying heart conditions (Freemantle et al, 1999; Zylla et al, 2024). The most notable evidence comes from the Cardiac Arrhythmia Suppression Trial (CAST), which found that the use of flecainide and encainide, class I antiarrhythmics, resulted in a higher mortality rate than placebo in patients with myocardial infarction (post-MI). Specifically, the trial revealed that these drugs could actually precipitate fatal arrhythmias, leading to the early termination of the study owing to safety concerns (CAST Investigators, 1989). While the risks are lower for β-adrenergic receptor blockers (class II antiarrhythmic drugs), they must still also be used judiciously, particularly in patients with severe cardiac dysfunction. In some cases, especially at high doses or in patients with severe heart failure, β-blockers can cause excessive bradycardia, hypotension, or heart block, potentially leading to adverse outcomes, including an increased risk of arrhythmias (Waldo et al, 1996; Freemantle et al, 1999; Dondo et al, 2017). Despite such risks, β-adrenergic receptor blockers are still widely used because their overall benefit in reducing SCD and improving survival in heart failure and post-MI patients, which often outweighs their risks (Yndigegn et al, 2024). The abovementioned considerations highlight the need for careful patient selection and monitoring under antiarrhythmic therapy.

III. Antiarrhythmic therapy development pipeline: the last 10 years

It has been estimated that the average cost of a traditional drug discovery pipeline is $2.6 billion (US dollars), and a complete traditional workflow can take over 12 years (Mohs and Greig, 2017). Unfortunately, the number of novel antiarrhythmic targets and agents in the development pipeline has decreased substantially during the last few decades owing to conceptual, regulatory, and financial considerations (Saljic et al, 2023). We have analyzed the AAD therapy development over the last 10 years using data extracted from www.clinicaltrials.gov (Fig. 1). Alarmingly, the total number of interventional clinical trials encompassing preclinical, I, II, III, and IV phases for the treatment of arrhythmias is significantly lower than in other areas of medicine. Specifically, only 440 studies have been completed and/or were under development in the last decade (Fig. 1A). This is striking because SCD and arrhythmia represent a major worldwide public health problem, accounting for 15%–20% of all deaths (Srinivasan and Schilling, 2018). Approximately half of those 440 studies are in phase IV or pharmacovigilance stage looking for side effects caused over time after approval and marketing (Fig. 1B). Studies in early stages such as preclinical and phases I and II show much lower percentages, supporting the fact that there is limited innovation in the field of antiarrhythmic therapy. Within the different types of interventions, it can be observed that drug development, without other types of interventions, leads with about 74%, followed very far behind by device development (7.95%). While other areas like biologic medicine and gene therapy are growing rapidly, in this context they account for 1.14% and 0.45%, respectively, without combination with other types of interventions (Fig. 1C). We also analyzed clinical trials focusing exclusively on pharmacological interventions (325 studies). As shown in Fig. 1D, most of the studies (33.53%) are dedicated to clinical research and development of anticoagulant or antiplatelet therapy aiming to mitigate the risk of cerebrovascular accident (stroke), as frequently occurs in patients with AF. In addition to these strategies, 7.69% of clinical trials aim to explore improvements related to pharmacology during cardiac surgery, mainly to advance ablation techniques, improve cardiac device implantation (pacemaker, defibrillator, cardiac resynchronizer, and holters), and prevent or treat postoperative AF. For instance, 41% of all clinical trials are focused on palliating secondary effects of arrhythmias and avoid comorbidities after surgery. Other clinical trials (19%) aim to investigate whether traditional AADs or other known agents are more effective before or after electrical therapy or in combination with other AADs. Yet, other trials are focused on different formulations, doses, routes of administration, or small modifications in structure. As discussed in detail further in section *Exploring drug repurposing for arrhythmia treatment*, drug repurposing, also known as repositioning or reprofiling, has played a key role in the history of antiarrhythmic drugs. Drug repurposing still occupies an important place in antiarrhythmic intervention, constituting almost approximately 30% of the clinical trials during the last 10 years, as shown in Fig. 1D. Many of these repurposing drugs are indicated for cardiovascular diseases (eg, hypertension, diabetes, cardiomyopathies, and angina), which have been demonstrated to have an antiarrhythmic effect or to palliate the secondary arrhythmias induced by these diseases. It is impressive that all clinical trials reported in the last 10 years on antiarrhythmic therapies are not disease specific, invasive, or only focused on palliating secondary effects or comorbidities. Critically, only 8.61% of all studies aimed at developing innovative molecules that specifically target each type of arrhythmia, highlighting those destined to AF, long QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT), bradycardia, and ventricular arrhythmia (Fig. 1D). Most of these innovative drugs are designed to modulate specific cardiac ion channels like voltage-gated potassium, sodium, and calcium channels and sarcoplasmic release (RyR) channels (Fig. 1D). Most are selective for modulating a single ion channel: for example, F373280, a new therapy based on docosahexaenoic acid delivery, for the maintenance of sinus rhythm after electrical cardioversion; BMS-919373, a highly functionalized quinazoline, and potent IKur blocker used to treat atrial fibrillation; eleclazine, an inhibitor of the late cardiac sodium current, is being tested for ventricular tachycardia and LQTS, specifically LQTS type 3 (Bacic et al, 2017; El-Bizri et al, 2018); AP30663, a small conductance Ca^{2+}-activated K^+ (I_{KCa}) channel blocker shown to prolong the atrial effective refractory period and convert AF into normal sinus rhythm (Gal et al, 2020); nasal spray of etripamil (a calcium channel blocker) is being investigated for the acute reduction of rapid ventricular rate in patients with symptomatic AF or for the conversion of paroxysmal supraventricular tachycardia (Stambler et al, 2022; Abuelazm et al, 2023; Camm et al, 2023); ARM210 (also known S48168) is a potential disease-modifying therapy for CPVT as it repairs leaky RyR2 channels (Marks, 2023). Some compounds have multichannel blocking effects, like the intravenous HBI-3000 studied for the conversion of recent onset AF (Chen et al, 2019; D. Guo et al, 2011). Other compounds do not directly target ion channels but do so through secondary mechanisms: oral LQT-1213 (serum glucocorticoid inducible kinase 1) indicated for congenital LQTS type 1, 2, or 3; CRD-4730 (Ca^{2+} or calmodulin-dependent protein kinase II) evaluated in participants with CPVT or congenital heart defects; and OMT-28 (epoxyeicosanoid synthetic analog) tested in patients with persistent AF (Berlin et al, 2020; Giannetti et al, 2023; Kim et al, 2023). Yet, other drugs such as OPC-108459 and HIP2001 and administered in patient with paroxysmal and persistent AF, and oral CARDIX 101 under development for the treatment of bradycardia, have unclear or unknown mechanisms or the mechanism has not been disclosed as in the case of HSY244 for the treatment of AF (Linz et al, 2024). Natural products (1.23%) have also been tested as new drugs, such as Freeze-Dried California Table Grape, Wenxin Granules, DH001

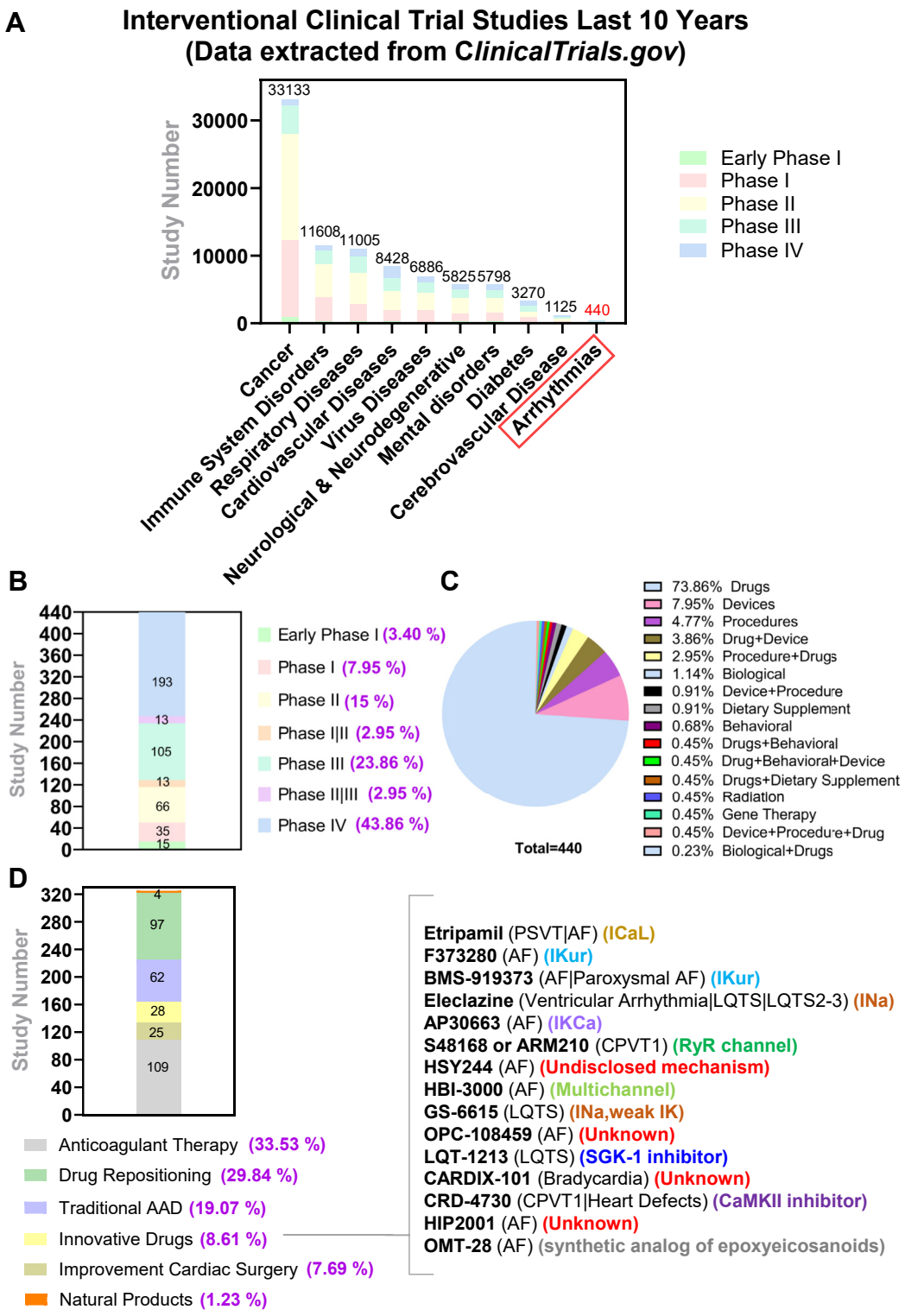

Fig. 1. Interventional clinical trials in the last 10 years. (A) Total number of interventional clinical trial studies in different clinical phases over the last 10 years, extracted from *ClinicalTrials*.gov. (B) Total number of interventional clinical trial studies for arrhythmia treatment. (C) Different types of interventions for all clinical trial phases from (B). (D) Pharmacological interventional clinical studies for arrhythmia treatment. CaMK, Ca^{2+} or calmodulin-dependent protein kinase; POAF, postoperative atrial fibrillation; PSVT, paroxysmal supraventricular tachycardia; SGK, serum glucocorticoid inducible kinase 1.

(active monomer from traditional Chinese medicine), and Tongmai Yangxin Pill (TMYXP) (Dong et al, 2017; Shi et al, 2021; Liu et al, 2022). However, their molecular mechanisms are unclear, and they are not disease specific.

The findings extracted from Fig. 1 unequivocally indicate that the pursuit for advances in antiarrhythmic therapies may be dwindling when juxtaposed with the progress observed in other realms of scientific research. The data underscore the limited

impact of innovative drugs focused on the future antiarrhythmic pipeline and highlight the comparatively significant advances in the field of anticoagulation associated with arrhythmias and drug repurposing. These observations prompt a reevaluation of research priorities, urging a redirection of efforts toward areas that show more promise for discovery and development of novel antiarrhythmic drugs. Altogether, our compilation highlights the slow progress in advancing improved antiarrhythmic therapies and underscores uncertainties regarding future developments in this field. Consequently, developing new experimental and technological approaches is highly desirable and particularly urgent to help overcome limitations. In the following sections, we discuss the limitations of antiarrhythmic drug discovery and how new tools can help accelerate progress.

IV. Human-induced pluripotent stem cell technology

Human-induced pluripotent stem cell (hiPSC) technology may revolutionize the field of biomedical science allowing a "clinical trial in a petri dish" (Takahashi and Yamanaka, 2006; Takahashi et al, 2007; Yamanaka, 2020). In the case of human cardiac diseases, the use of stem cell technology in preclinical experimentation supposes a significant advance. Heart tissue from human donors is difficult to obtain and does not regenerate, which has limited the development and discovery of drugs. Although the use of murine models for human diseases is standardized in biomedical research, interspecies differences make the study of arrhythmias difficult to translate to the human (Doncheva et al, 2021). The HR of mice is 10 times faster than the HR of humans, and the AP characteristics are vastly different because of the lack of functional expression of some human ion channels in the mouse (Edwards and Louch, 2017). On the other hand, heterologous cell lines (HEK, CHO, and HL-1) lack the functional and structural characteristics of human cardiomyocytes and present aneuploidies (Baik and Lee, 2017; Li and Zhu, 2022). hiPSCs can be generated in nearly unlimited quantities. They can be cultured for long periods and cryopreserved. In addition, they are readily available in the market and can be differentiated into multiple cell lineages, including human-induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs) (Shafa et al, 2018). In addition, they can model human diseases better than other platforms like immortalized human or transgenic cell lines and can be reprogrammed directly from patients' own cells. In addition, the ability to differentiate hiPSCs into hiPSC-CMs provides a unique platform to study cardiac diseases without cell limitation. Notably, hiPSC-CMs express a collection of ion channels that enable them to generate cardiac-like APs. This offers huge advantages over heterologous cell systems in which a single ion channel is tested (Rogers et al, 2016; Cunningham et al, 2019). Moreover, iPSC-CMs can form electrically coupled monolayers and engineered cardiac tissue constructs that can be used to quantify electrical impulse propagation and study mechanisms of re-entrant arrhythmias (Jimenez-Vazquez et al, 2022).

During the last decade, attempts have been made to standardize the use of hiPSC-CMs for drug discovery as well as the assessment of cardiotoxicity, which is a primary risk factor in cancer drug development (Sharma et al, 2018; Gintant et al, 2019). hiPSC-CMs are central to the Comprehensive in-vitro Proarrhythmia Assay (CiPA) consortium. CiPA was established in 2013 as a step to confirm the results of in vitro and in silico tests on drug effects on multiple cardiac ion channels and the cardiac AP. Such effects are focused on proarrhythmia and the potential to generate Torsade de Pointes (TdP), a polymorphic ventricular tachycardia that can lead to SCD (Sager et al, 2014; Strauss et al, 2019). It has the support of regulatory agencies, academic laboratories, and pharmaceutical companies (QandA ICH-Guideline E14/S7B Clinical and Non-Clinical Evaluation EMA Document; https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-e14s7b-clinical-and-nonclinical-evaluation-qtqtc-interval-prolongation-and-proarrhythmic-potential-questions-and-answers-step-5_en.pdf). New technologies as artificial intelligence (AI) and deep learning are also helping to discern the risk of drug-induced arrhythmia by analyzing features of in vitro AP recordings in hiPSC-CMs that correlate with clinical arrhythmia manifestations (Serrano et al, 2023). Ideally, some of the new candidate drugs would move to start the different phases of clinical trials directly after checking their effectiveness and toxicity in hiPSCs (Sharma et al, 2018). However, even the most common hiPSC-CMs in vitro assays have important limitations that limit their full acceptance by regulatory agencies, as well as scientific publications. Such limitations include the hiPSC-CMs' lack host immune components and their fetal/neonatal cardiomyocyte-like phenotypes, with paucity of important ion channels (eg, inward rectifying potassium [I_{K1}]), which make them unable to fully recapitulate drug effects on adult human cardiomyocytes (da Rocha et al, 2017; Yang et al, 2022b).

Despite the aforementioned limitations, hiPSC-CMs are a promising tool for drug discovery. In addition, the use of this technology not only is limited to testing new drugs but also applies to disease modeling, disease phenotype anticipation, re-engineering previous drugs with fewer side effects and personalized medicine (Correia et al, 2023). As reviewed recently by other authors (Matsa et al, 2016; Musunuru et al, 2018; Pourrier and Fedida, 2020), advances in hiPSC-CM research have provided a platform to effectively study patient-specific heart disease in vitro. The use of patient-specific hiPSC-CMs may be useful for basic science investigations, as well as for patient-specific drug screening and personalized therapy. Several studies have focused on testing new antiarrhythmic drugs in hiPSC-CMs with promising results. For example, Dago et al (2022) showed that the use of dapagliflozin and empagliflozin could be used as a new class of antiarrhythmic in heart failure by increasing sodium (I_{Na}) and I_{K1} currents. Apart from testing new drugs, the use of hiPSC-CMs is helping in re-engineering existing drugs with better therapeutic results and less side effects, as is the case of mexiletine in LQTS3 (McKeithan et al, 2020).

Human iPSC-CMs serve not only to study diseases in patients' own cells. Gene editing tools like CRISPR/Cas 9 (Han et al, 2023), transcription activator-like effector nucleases, and zinc-finger nucleases (Hockemeyer et al, 2011; Wang et al, 2014; Li et al, 2015) are helping to develop new disease models and to generate isogenic hiPSC-CMs for experimental controls (Guo et al, 2019). Wang et al (2014) reproduced patient phenotype of LQTS by introducing dominant negative mutations in KCNQ1 by zinc-finger nucleases (. Gene editing helps also in the discovery of new treatments for cardiac pathologies. Nowadays, variants of uncertain significance (VUSs) are emerging as an important challenge in clinical genetics, with enormous implications for precision medicine (Fatkin and Johnson, 2020). VUSs show no evidence of pathogenicity (Richards et al, 2015), but some health providers describe VUSs as likely related to monogenic cardiovascular disorders such as cardiomyopathies and rhythm disorders even though they do not meet clinical criteria (Muller et al, 2020). hiPSC-CMs are thus a good platform to study such variants. Garg et al (2018) observed that a VUS in *KCNH2* (LQTS2) was pathogenic. The study of the molecular mechanisms of these pathogenic variants in the same or different genes, help scientists to observe common mechanisms to treat different diseases. Mutations in different genes or even different mutations in the same gene can have different consequences at the molecular level but still have the same clinical outcome, for example, cardiomyopathy (Spielmann et al, 2022). Patient-specific iPSCs offer the opportunity to dissect mechanisms directly relevant to the patients' mutations (Campbell et al, 2015; Ma et al, 2018). By virtue of having a perfectly matched genetic background,

patient-specific iPSCs can provide a model system that integrates all genomic loci involved in the response to medication (Carcamo-Orive et al, 2017). This is crucial because antiarrhythmic therapy depends on the specific type of arrhythmia, its underlying cause, and individual patient characteristics. Another great advantage of using patient-specific iPSC-CMs is that they are an unlimited resource to apply single-cell omics assays when it is almost impossible to obtain cardiac tissue from patients. This opens a new world of treatments and breaks out the traditional method of drug testing in the patient by trial and error. With patient-specific iPSC-CMs, one can test the cell response to different types of drugs at wide ranges of drug concentration (Theodoris et al, 2021). Moreover, using omics, one can identify new biomarkers and targets for disease treatment. The combination of any given patient's unique clinical, genomic, proteomic, and in vitro cellular characteristics obtained from hiPSC-CM experiments may help the clinician in making decisions regarding diagnosis, treatment, and prevention of human diseases, providing a personalized treatment (Perry et al, 2021).

However, although the above-described approaches could lead to major advances, using iPSC-CM has potential limitations that must be considered. An immature phenotype is the principal concern. These cells are proarrhythmic owing to their immature electrophysiological phenotype and cell structure (Goversen et al, 2018). In addition, the level of hiPSC-CMs maturation determines drug responsiveness in preclinical cardiotoxicity and proarrhythmia screening (da Rocha et al, 2017). Moreover, the time to reprogram cells and the mix of cell types after differentiation (ventricular, atrial, and nodal cells) are a concern. In the past years, several methodologies have been developed to solve their fetal-like phenotype. Some methods include long-term culture (Kamakura et al, 2013; Seibertz et al, 2023), addition of hormones (Parikh et al, 2017), and fatty acids to cell culture media (Yang et al, 2019; Feyen et al, 2020), cell coculture, use of extracellular matrix (ECM) or biomaterials, and mechanical or electrical stimulation (Ronaldson-Bouchard et al, 2018), and new platforms that try to recapitulate several of these methods are emerging. These new platforms also include coculture of different cell types, interactions of cells with the microenvironment (cell-cell and cell-ECM interactions), and physiological cues, facilitating more translational studies owing to their higher similarity to the adult heart (Beauchamp et al, 2020; Schmidt et al, 2023). hiPSC-CM monolayers are generally less mature than 3-dimensional (3D) constructs; however, they do show some promise in enabling the study of AP propagation and re-entrant arrhythmias (da Rocha et al, 2017). On the other hand, it is expected that the new 3D platforms would solve problems seen with 2-dimensional (2D) constructs. Some engineering heart tissues are 3D scaffolds formed by hiPSC-CMs and ECM or biomaterials as hydrogels (Eder et al, 2016). An example is the commercialized Biowire, where engineering heart tissues are electrically stimulated, enabling cardiac maturation, with good results in cardiotoxicity testing (Nunes et al, 2013; Feric et al, 2019). Microtissues are 3D models containing hiPSC-CM cocultured with fibroblasts and endothelial cells. Giacomelli et al (2020, 2021) have generated these cardiac microtissues with excellent results probing them in the study of arrhythmogenic cardiomyopathy and LQTS2. However, microtissues cannot self-organize, they do not contain vasculature and cannot recapitulate the developing heart. In contrast, organoids are small 3D self-organizing cellular aggregates containing multiple cell types that represent more structurally accurate models of the human myocardium. Some authors have used organoids for drug screening owing to their similarity to an adult heart and because they contain cells from the 3 germ layers (Mills et al, 2019). Beyond organoids are the assembloids, which can combine organoids with cells from diverse tissue lineages or from artificially assembling multiple organoids (Campostrini et al, 2021; Ng et al, 2022). Assembloids reproduce the interactions among multiple subregions and cell types. In cardiovascular diseases, assembloids could advance targeted, tissue-specific drug treatment in scenarios where drugs differentially affect subsections of an organ or tissue, for example, the atria and the ventricle (Schmidt et al, 2023). However, the use of this platform has limitations as most organoid types resemble early stages of human heart during morphogenesis. Finally, nowadays, the use of organ-on-a-chip (OoC) technology that allows the interaction of different cell types is taking relevance (Chen et al, 2013; Kim et al, 2014; Picollet-D'hahan et al, 2021). OoCs are designed for the growth of multiple organ-specific cell types in a fully integrated system, often with different cell types cultured in separate compartments that are interconnected via perforated membranes or juxtaposed microchannels. The approach allows coculturing multiple cell lineages that underlie organ function and enables fluid flow, and in some cases, mimicking stretch to further simulate the in vivo environment. This allows the administration of drugs to the blood channel of an OoC, while observing the effects on an adjacent tissue channel, recapitulating the in vivo environment and adding power to the model (Azizgolshani et al, 2021). However, OoCs are still under development and their primary focus resides on drug cardiotoxicity screening (Zhao et al, 2020). An upgrade version of OoCs are the organoids in an OoC or body-on-a-chip. This revolutionary technique combines 3D models of organoids with a fully integrated and connected system, where changes can be studied in a more complex microenvironment. A good example is the exposure to clomipramine (a tricyclic antidepressant) in an OoC, composed of a liver organoid chamber and heart organoids in the bottom chamber. Heart organoids presented impairments in cell viability, cardiac beating, and calcium activities, suggesting the hepatic metabolism-dependent cardiotoxicity of clomipramine (Yin et al, 2021). This impressive multiorganoids-on-a-chip system can recapitulate the complex process of drug metabolism at the multiorgan level. Skardal et al (2020) developed a 3-tissue organoid-on-a-chip platform to test a multipanel of FDA-recalled drugs, demonstrating that some of these drugs caused toxicity in liver and heart. Moreover, they went a step further to develop a 6-tissue body-on-a-chip platform where they observed that capecitabine, an anticancer drug, must be metabolized in the liver to become active. This effect generated secondary effects in other organoids as cardiac and lung constructs.

V. Multiomics

The use of omics has also contributed to revolutionize the field of drug discovery and drug testing (Nguyen et al, 2022). In recent years, genomics, transcriptomics, proteomics, and metabolomics platforms have been incorporated to the study of complex interactions in biological systems. Moreover, these new tools break away from the traditional concept of "1 drug, 1 target," that is, a single molecular target with high selectivity to avoid other biological off-targets. The approach is useful in monogenic diseases, where traditionally 1 gene mutation has implied 1 phenotype. The majority of diseases are multifactorial or polygenic and need multitarget drugs to be treated (Bolognesi and Cavalli, 2016). Each of the different omics is focused on a regulatory process. Thus, genomics study DNA sequences and allelic variants in organisms by sequencing. Genomics also includes epigenomics, which studies how the genome is modified such that the DNA sequence does not change, but the organism's observable traits do (Westerman et al, 2020). Epigenomics can also show the functional interaction among regulatory genes and coding and noncoding regions. A step further, with and additive effect to these techniques, is the study of

RNA by transcriptomics. Transcriptomics can give information about RNA structure, stability, variants, and alternative splicing (Litviňuková et al, 2020). Following the central molecular biology dogma, after RNA, translation occurs. A more complex omic is proteomics. Proteomics allows the study of changes in the levels and posttranslational modifications of a protein in the organism (Alvarez-Franco et al, 2021; Liu et al, 2020). Finally, metabolomics, which is gaining relevance in recent years, studies complex interactions in the different cell compartments. Metabolites are the end products of the cell and are involved in processes like degradation, enzyme kinetics, transport, and secretion (Zhang et al, 2022). However, although all omics are specialized in a part of a biological system, they are focused on the generation of complex networks of genes, transcripts, proteins, or metabolites to characterize cell types and study normal and pathological conditions in a quantitative and qualitative manner.

All omics are based on new technical platforms as next-generation sequencing, RNA-sequencing, chromatin-immunoprecipitation sequencing and DNA methylation sequencing, single-cell sequencing, liquid chromatography, and mass spectrometry. All these new applied omics techniques generate large amounts of data that need to be process and registered in databases. Several articles have extensively reviewed the different types of databases (Matsa et al, 2016; Paananen and Fortino, 2020; Satam et al, 2023). Other omics that are emerging in recent years are lipidomics (full characterization of lipids), glycomics (study of glycans produced by cells under specified conditions), and glycoproteomics (study of glycoproteins, glycan structure, and protein identity and glycosilations). Nowadays, omics data help in pharmaceutical research. In the case of arrhythmogenic diseases, the combination of omics with stem cell technology has helped in diagnosis, drug discovery, and treatment of diseases. As such, genomics helps in the identification of mutations responsible of genetic disorders or genetic polymorphisms and can predict prognosis, severity, and drug responsiveness (Wilson et al, 2015). Genomics also helps in understanding pathogenesis (eg, genome-wide association study databases), patient stratification, and discovery of putative drug targets or assessing the efficacy and toxicity of drugs at the genetic level. For example, the identification by genome-wide association studies of new genes involved in AF (Liu et al, 2020). Another example is the use of genomics in Brugada syndrome, which has helped in the development of a genetic risk score, owing to the different *SCN5A* variants related to disease severity (Wijeyeratne et al, 2020). Transcriptomics also serves as a platform for drug discovery and drug evaluation, as well as to predict adverse drug target effects. Such is the case of the use of an agonist of platelet-derived growth factor that decreased arrhythmia incidence in dilated cardiomyopathy caused by a laminin A gene mutation (Lee et al, 2019a). However, transcriptomics goes a step further because it can decipher disease mechanisms and the mode of action of drugs (Alexander-Dann et al, 2018).

Although transcriptomics is the most readily used single-cell omics—level analysis, proteomics and metabolomics are gaining force in the discovery and toxicity evaluation of drugs, as well as identifying new biomarkers. Proteomics studies posttranslational processes and the interactome, helping identify molecular pathways like the ProteomicsDB database that also helped in drug target identification (Samaras et al, 2020). Proteomics data also provide information for testing drug targeting efficacy and safety, as well as protein toxicology. Chemoproteomics is a new technology that combines mass spectrometry proteomics with chemical methods. In chemoproteomics, small molecules bind to protein targets, indicating the amount of drug necessary for binding and what therapeutic effect it will produce. Moreover, the approach can assess off-target interactions by determining drug selectivity (Jones and Neubert, 2017). Finally, metabolomics has similar advantages as proteomics, but adding drug target discovery. An example of these omics applied to the arrhythmogenic field is the identification of proteomic and metabolite changes in Brugada patients with respect to control patients, identifying novel disease biomarkers (Di Domenico et al, 2013). Although all omics are useful, focusing on a single omic technique cannot elucidate the entire biological response to drug treatment. Then, the combination of different omics in multiomics or high-throughput technologies entails a better understanding of drug-related mechanisms (Nguyen et al, 2022). The birth of AI and machine learning (ML) is also helping in processing the huge amounts of data obtained from omics. However, the results derived from the use of techniques should be complemented necessarily by functional studies to determine how individual genetic variants affect drug responses. Pharmacogenomics is thus related to personalized medicine because each patient is unique owing to personal single-nucleotide polymorphisms (SNPs). These may induce patient variability in drug efficiency as described for the various degrees of severity of Brugada syndrome (Di Domenico et al, 2013; Wijeyeratne et al, 2020). Most AADs that target cardiac repolarization by blocking K^+ current (I_{Kr}; flecainide, amiodarone, dronedarone, and sotalol) cause drug-induced TdP (Behr and Roden, 2013).

The risk of developing LQTS or TdP can be influenced by genetic factors, particularly SNPs that affect the function of cardiac ion channels: for example, drug-induced LQTS caused by sulfamethoxazole antibiotic medication. Sesti et al (2000) reported that a SNP in the *KCNE2* gene encoding MiRP1 (T8A-MiRP1), a subunit of the human ether-a-go-go-related gene (hERG) channels that has been associated previously with inherited LQTS, underlies genetic predisposition of sulfamethoxazole-induced LQTS. Importantly, I_{Kr} in a patient with T8A-MiRP1 SNP was normal at baseline but at least 4-fold more sensitive to therapeutic levels of sulfamethoxazole than wild-type channels (Sesti et al, 2000). Sulfamethoxazole speeded deactivation (closure) only of I_{Kr} formed with T8A-MiRP1 SNP (Sesti et al, 2000). Hence, patients carrying these genetic variants may be at a higher risk of developing drug-induced LQTS when exposed to sulfamethoxazole, as the drug's effect on cardiac repolarization is amplified owing to their underlying genetic predisposition. This illustrates the importance of pharmacogenomics in predicting adverse drug reactions and tailoring treatments to individual genetic profiles. For more examples, Alexander-Dann et al (2018) provide a summarized table of the different drugs and genes involved in the pharmacogenomics of antiarrhythmic drugs.

VI. Computational screening for accurate development of new chemical compounds

Advancements in computational modeling in drug discovery have revolutionized the pharmaceutical industry, accelerating the identification and development of novel therapeutics. Traditional drug discovery methods are time-consuming and expensive, but computational modeling expedites the process by predicting molecular interactions, identifying potential drug targets, and optimizing compound structures (Sliwoski et al, 2014; Sadybekov and Katritch, 2023). The process involves algorithms, simulations, and databases to simulate molecular interactions, predict compound behaviors, and evaluate their efficacy and safety. This reduces considerably the need for extensive laboratory testing and significantly decreases the time and resources needed for drug discovery and basic science. Initially, researchers input structural data of target molecules or biological systems into computer models. Molecular docking predicts how small molecules (potential drugs) interact with target proteins, simulating their binding and affinity (Kitchen et al, 2004; Gioia et al, 2017; Kontoyianni, 2017). Using

molecular docking software, the ligand's 3D structure is virtually fitted into the binding site of the target molecule, exploring various orientations and conformations. The software evaluates and scores these ligand-protein interactions based on factors like binding energy, complementarity, and predicted stability of the complex. Docking studies help identify potential drug candidates by assessing their likelihood to bind to the target and modulate its activity. They guide medical chemists by suggesting structural modifications to optimize binding affinity and specificity. However, docking models have limitations like considering only the static structure of proteins and simplifying the complexities of molecular interactions in living systems (Kolb et al, 2012). Nevertheless, the combination of new and feasible molecules by docking with molecular biology and STEM (science, technology, engineering and mathematics) technology will allow the discovery of new drugs. Further, molecular dynamics simulations allow researchers to understand the dynamic behavior of druggable molecules and their interactions with biological targets at a level of detail that was once unimaginable (De Vivo et al, 2016; Kuzmanic et al, 2020; Ahmed et al, 2023). This insight aids in designing more effective and specific drugs with fewer side effects. However, as before, integrating experimental proof is crucial to validate and enhance the reliability of the predictions generated through in silico docking studies. For instance, research indicates that flecainide and propafenone increase I_{K1} by reducing polyamine blocking of the strong inward rectifier potassium channel Kir2.1 (Caballero et al, 2010; Gómez et al, 2014). Structural modeling combined with experimental validation has illustrated that both flecainide and propafenone bind to Kir2.1 through specific interactions with Cys311. Such an interaction has facilitated the identification of the pharmacophore associated with drugs that enhance Kir2.1 function (Caballero et al, 2010; Gómez et al, 2014). Similarly, investigations at the atomic scale have delved into the structure-based molecular mechanisms of AADs. Researchers used Rosetta structural modeling, docking techniques, and molecular dynamics simulations to scrutinize the interactions of AADs like flecainide, lidocaine, and ranolazine, with the human voltage-gated sodium channel ($Na_V1.5$) (Meiler and Baker, 2006; Bender et al, 2016). Those calculations unveiled pivotal drug-binding sites within the pore lumen, capable of simultaneously accommodating up to 2 drug molecules. Interestingly, molecular dynamics simulations further elucidated a hydrophilic access pathway through the intracellular gate and a hydrophobic access pathway via a fenestration situated between DIII and DIV of $Na_V1.5$ (Nguyen et al, 2019). The assessment of AP within an in silico modeling framework has also proven to be a potent tool for early detection of drug-induced proarrhythmic risk. This evaluation showcases its efficacy in discriminating torsadogenic compounds that impact the AP duration across isolated endocardial, midmyocardial, and epicardial cells, along with inducing QT prolongation in human ventricular AP models (Romero et al, 2018).

Quantitative structure-activity relationship (QSAR) models can be integrated in the workflow analysis of chemical and biological properties to forecast a compound's activity (Lipinski et al, 2001). Specially, QSAR models decipher the intricate relationship between a compound's structure and its biological activity through computational analysis. By scrutinizing various chemical descriptors—molecular size, shape, electronegativity, and more—alongside biological properties like receptor affinity or enzyme inhibition. QSAR models predict how structural modifications impact a compound's effectiveness or toxicity (Bradbury, 1995). These models use statistical techniques to generate equations correlating the chemical structure's quantitative features with the observed biological activity, facilitating the prediction of untested compounds' behavior. In silico methods accelerate drug discovery by reducing the number of compounds requiring physical testing, optimizing lead compounds, and predicting potential side effects. However, they rely heavily on the quality of input data and model accuracy (Holzinger et al, 2019). Continuous advancements in computational power and algorithms enhance the precision and efficiency of these techniques, reshaping the landscape of pharmaceutical research and expediting the development of novel therapeutics. For example, QSAR plays a pivotal role in addressing drug-induced TdP risks (Frid and Matthews, 2010; Broccatelli et al, 2012; Das et al, 2023). QSAR models use large datasets, such as those resulting from compulsory hERG screening, to establish correlations between molecular structures and biological activities. QSAR aids in predicting IC_{50} values for hERG blockade, providing valuable insights into the potential cardiotoxic effects of drugs (Wacker and Noskov, 2018; Choi et al, 2020). However, the evolution beyond traditional QSAR techniques, with modern ML algorithms, particularly eXtreme Gradient Boosting (XGBoost), demonstrates superior accuracy in determining hERG blockade and paves the way for more advanced predictive models in drug development and safety assessment (Wacker and Noskov, 2018; Yang et al, 2023).

AI and ML have become pivotal in revolutionizing drug development in the future (Sarkar et al, 2023). These are technologies based on vast datasets and advanced algorithms; they predict biological activities and expedite drug discovery, potentially reducing costs and timelines. Through pattern recognition and analysis, AI models can sift through massive amounts of biological, chemical, and clinical data to identify potential drug candidates, predict their efficacy, optimize molecular structures, and even anticipate adverse effects (Zhu et al, 2022b; Mullowney et al, 2023; Yang et al, 2023). These models learn from diverse data sources (eg, omics information, protein structures, drug interactions, and patient data) to suggest promising compounds, assisting in virtual screening, and predicting a compound's potential activity against a target. For instance, AI and data mining helps in all omic data processing, contributing to the new field of pharmacogenomics and personalized medicine, as has been done in type 2 diabetes (Allesøe et al, 2023). Virtual screening, empowered by ML and deep learning, rapidly analyzes vast chemical libraries to identify potential candidates for further experimental validation processes to ensure the efficacy and safety of identified compounds. Its integration into drug development pipelines has revolutionized early-stage screening, offering a more efficient pathway for identifying promising drug candidates (Chan et al, 2019; Sarkar et al, 2023). Therefore, these technologies hold immense promise, despite facing challenges like data quality representation and ethical considerations, reshaping drug development and potentially expediting the delivery of safer and more effective treatments to patients worldwide (Vora et al, 2023b). In summary, the integration of computational modeling techniques has greatly enhanced the efficiency and effectiveness of drug discovery, promising a future where the development of new therapies is not only faster but also more precise and tailored to individual patient needs. However, computational technologies are not understood alone. They need validation in biological samples that test functional outcomes like electrophysiology in the case of arrhythmogenic diseases and drug therapy.

VII. High-throughput screening electrophysiologic platforms

Drug discovery in the cardiovascular diseases field is constantly changing and has been evolving with emerging technologies and methodologies to meet challenges and needs. The recent evolution of HTS is playing an essential role in drug discovery for antiarrhythmic therapy by contributing to increase success rates in the

identification and development of effective and safe potential drug candidates (Wilson et al, 2015; Bunch et al, 2023; Satam et al, 2023). The use of HTS has revolutionized traditional drug development in cardiac diseases by expediting the efficient identification of lead compounds, optimizing their properties, and enhancing our understanding of the complex mechanisms underlying cardiac arrhythmias and potential drug interactions (da Rocha et al, 2017; Bruyneel et al, 2018). A good example is the study by Sharma et al (2017), where using hiPSC-CMs and applying various HTS assays, they identified a signaling pathway by which different tyrosine kinase inhibitors, used in oncology, produce cardiovascular side effects ranging from induced arrhythmias to heart failure. Increased cardioprotective signaling of this pathway identified with exogenous insulin or insulin-like growth factor 1 enhanced the viability of hiPSC-CMs during cotreatment with cardiotoxic tyrosine kinase inhibitors. This approach provided the unexpected insight that cardiotoxicity and arrhythmias induced by certain anticancer drugs can be alleviated via cardioprotective insulin/insulin-like growth factor signaling (Sharma et al, 2017). HTS allows for the rapid and simultaneous screening of large compound libraries against multiple targets implicated in the complex mechanisms of arrhythmogenesis. Thus, HTS accelerates the identification of compounds with potential antiarrhythmic properties fostering innovation in drug discovery beyond traditional targets (Wells et al, 2019). HTS significantly reduces the time and resources required by automating the screening process into a short period. For example, Glazer et al (2020) used a high-throughput automated patch-clamp system to study the function of the 83 variants in *SCN5A* gene associated with Brugada syndrome. They reclassified the variants with functional data expressed in HEK cells (Glazer et al, 2020). This efficiency is particularly valuable in the context of arrhythmia stratification and antiarrhythmic therapy, where rapid intervention may be critical (Al-Khatib et al, 2018). For example, a study established a high-throughput drug testing system on 13 different compounds using human iPSC derived atrial-like myocytes for drug discovery in AF (Honda et al, 2021). HTS assays facilitate the optimization of lead compounds, which can then be further investigated for enhanced doses, efficacy, and selectivity, improving the candidate's therapeutic profiles. These assays can also be used to assess potential cardiotoxic effects early in the drug development process, helping to prioritize compounds with a lower risk of adverse cardiac effects (Krishna et al, 2021). HTS allows the use of patient-specific iPSC models, contributing to the development of personalized antiarrhythmic therapies that consider individual variations in drug response (del Álamo et al, 2016), as described in detail in previous sections. The complementary use of hiPSCs and HTS technologies offers great versatility and advantages for arrhythmia research: for example, one can validate the targets involved in the development of arrhythmias identified by omics, as well as test new drugs designed against these specific targets designed by computational modeling as explained in previous sections. Evidently, not only has HTS benefited greatly from hiPSC technology, but also many of these technologies have been designed specifically for application in hiPSC-CMs and not in animals or heterologous expression systems as previously shown. Priority is now being given to the HTS technologies using automated electrophysiological platforms. Automated patch-clamp systems can rapidly screen many genetic variants and/or compounds and analyze their effects on multiple ion channels aiding in the identification of potential candidates (Walsh, 2015; Obergrussberger et al, 2021). For example, a study was able to test the effects of 26 drugs and 3 drug combinations on 2 iPSC-CM lines using high-throughput voltage-sensitive dye and microelectrode-array assays. Drugs were studied for the CiPA initiative, and results were compared with clinical QT prolongation and TdP risk (Blinova et al, 2017). Automated systems often allow for parallel recordings from multiple cells or channels simultaneously. This enables researchers to study the effects of compounds on different cell types or ion channels more efficiently (Obergrussberger et al, 2021). Moreover, automated patch-clamp systems offer various advantages with respect to manual patch-clamp, such as much faster experimental rates, consistency, reproducibility, continuous recording, user-friendly interfaces, customization, and flexibility to design experiments (Obergrussberger et al, 2021). For example, a study validated state dependence and subtype selectivity of reference calcium channel modulators (nifedipine, BAY K8644, verapamil, mibefradil, and pimozide) on human Cav1.2, Cav2.1, Cav2.2, and Cav3.2 channels using automated electrophysiology assays (Kuryshev et al, 2014). It is important to note that manual patch-clamp techniques still have their place, especially in situations where fine control, manual dexterity, or specific experimental conditions are crucial.

Noninvasive electrocardiographic imaging, which allows the reconstruction of high-resolution cardiac electrical activity maps, enables the assessment of drug-induced changes in cardiac electrophysiology (Rudy, 2013). Experimental cardiac optical mapping is the gold standard for measuring complex electrophysiology in ex vivo and in vitro heart preparations. Optical mapping uses voltage-sensitive or calcium-sensitive fluorescent dyes to visualize and measure the dynamics and drug-induced changes in electrical wave propagation on both the surface of an animal heart (Lee et al, 2019b) and hiPSC-CM monolayers (Liu et al, 2023). Microelectrode array platforms can monitor the electrical activity. In 2D iPSC-CMs monolayers, recording the field potential duration, an electrical signal similar to the ECG. This technique also provides insights into drug-induced changes in cardiac rhythms (del Álamo et al, 2016; Zhao et al, 2022). In addition, techniques for the analysis of electrophysiological function in cardiac organoids, 3D culture systems, and OoCs are being developed, which should provide more relevant insights compared with traditional cell culture models (Zhang et al, 2016). During the last decade, advanced automated microscopy techniques are been incorporated simultaneously to control and monitor the electrical activity and calcium handling of specific cardiac cell types for studying drug effects at cellular and subcellular levels (Huebsch et al, 2015; Dvinskikh et al, 2023). HTS generates large datasets that can be integrated with computational and bioinformatics approaches, providing a systems-level understanding of drug effects on cardiac electrophysiology. The integration of all such electrophysiological datasets with multiomics and bioinformatics approaches (ML algorithms and AI) can help identify potential antiarrhythmic therapies (Trayanova et al, 2021; Galappaththige et al, 2022; Sarkar et al, 2023). These emerging HTS electrophysiological technologies offer more precise, relevant, and efficient ways to evaluate the antiarrhythmic potential of new drugs, ultimately enhancing drug safety assessments and reducing the risk of adverse cardiac events during drug development and clinical use.

VIII. Exploring drug repurposing for arrhythmia treatment

Developing a new drug from scratch is a time-consuming and expensive process (Kale et al, 2022). To move forward, researchers have to consider and explore other approaches like drug repurposing. Drug repurposing involves identifying novel therapeutic uses for existing drugs outside their originally intended scope (Gelosa et al, 2020; Abdelsayed et al, 2022; Choudhury et al, 2022). The strategy offers a cost-effective alternative and leverages the extensive knowledge available about a drug's safety profile, pharmacokinetics, and mechanisms of action, potentially expediting the development process (Kale et al, 2022). Known safety profiles of

repurposed drugs can mitigate the risk of unexpected adverse effects. This advantage is particularly crucial in cardiac care, where the consequences of adverse reactions can be severe (Gelosa et al, 2020; Abdelsayed et al, 2022; Saljic et al, 2023). Moreover, repurposed drugs often possess mechanisms of action that can address multiple pathways involved in arrhythmogenesis. This multifaceted approach may enhance therapeutic efficacy compared with traditional single-target drugs (Wasim et al, 2023). In addition, HTS technology can also be used to screen existing drug libraries to identify compounds with unforeseen antiarrhythmic properties, facilitating drug repurposing strategies (Abdelsayed et al, 2022). There are several interesting examples of repurposed drugs for arrhythmias. For example, originally amiodarone was developed as an antianginal agent owing to its vasodilating effect; however, amiodarone was repurposed as an antiarrhythmic drug owing to its potent effect on various ion channels (Tavolinejad et al, 2019). It is now widely used to treat life-threatening ventricular arrhythmias. A derivative of amiodarone, dronedarone, was developed to reduce amiodarone's side effects while retaining its antiarrhythmic properties and is used in the management of AF (Vamos and Hohnloser, 2016; Pannone et al, 2021). Other examples are verapamil and diltiazem, calcium channel blockers, initially designed for hypertension and angina treatment. These drugs have shown efficacy in certain supraventricular arrhythmias by modulating calcium currents in the heart (Godfraind, 2017). A more recent example is ranolazine, which was developed initially as a metabolic modulator and approved as an antianginal agent; however, it was also identified as an inhibitor of the cardiac late Na^+ and hERG currents. The latter actions underlie ranolazine's antiarrhythmic effects on both supraventricular and ventricular arrhythmias. However, despite initial enthusiasm, ranolazine is only authorized as a second-line treatment in patients with chronic angina pectoris, notwithstanding its antiarrhythmic properties (Frommeyer et al, 2016; Shenasa et al, 2016; Rouhana et al, 2021). In summary, while drug repurposing holds great promise for arrhythmia treatment, challenges persist. Rigorous clinical trials are essential to establish the safety and efficacy of repurposed drugs in specific arrhythmia subtypes. Additionally, identifying suitable candidates for repurposing and understanding the precise mechanisms of action remain ongoing challenges.

IX. Gene therapy approaches

Recombinant adenoassociated viruses (rAAVs) have been successfully used as the selected vehicle for viral gene delivery in a wide range of clinical applications in multiple diseases (Mendell et al, 2021). In the last decades, AAV gene delivery progressed to FDA-approved clinical trials with a good safety profile and promising results in the treatment of genetic diseases such as hemophilia, lipoprotein lipase deficiency, inherited retinal disease, and spinal muscular atrophy (Mingozzi and High, 2011; Nathwani et al, 2011; Gaudet et al, 2013). However, the use of AAV gene therapy has limited success to prevent and treat cardiac disorder (Bass-Stringer et al, 2018; Ishikawa et al, 2018). Intracoronary infusion of AAV1 encoding SerCA2a (a phase IIb) failed to demonstrate beneficial results of AAV-based gene therapy in patients with heart failure (Greenberg et al, 2016). There are still numerous challenges that need to be resolved, such as the inability of rAAVs to effectively target specific tissues, pre-existing neutralizing antibodies in human populations, the wrong molecular targets or inadequate doses administered (Shen et al, 2022). However, the interest for clinical rAAV gene transfer approaches to treat various disorders in other tissues has propelled growing interest and progress in using rAAVs for cardiac disorders. Gene therapy has the potential to correct the underlying genetic defects that contribute to the development of arrhythmias rather than simply alleviating symptoms, offering long-term, precise, and targeted treatment (Bass-Stringer et al, 2018). However, gene therapy for cardiac arrhythmias is still in a research and development phase, and there are significant challenges to overcome before it can become a standard treatment. As described earlier, AAVs are one of the most widely used gene delivery systems; however, they present specific limitations for cardiac gene therapy (Ishikawa et al, 2018; Shen et al, 2022). AAV DNA capacity is limited to <4.8 kb for efficient packaging, which means that they can only carry and deliver genes of relatively small size. This is a major limitation for the treatment of arrhythmias because cardiac ion channel encoding genes are usually very large. Furthermore, achieving uniform, efficient, and specific transduction in cardiac cells can be challenging, and AAVs tend to accumulate in other tissues, especially liver, leading to adverse effects. CRISPR technology is therefore considered for gene therapy in certain instances, although it has its own limitations. Despite such limitations, AAVs remain a valuable option in cardiac gene therapy, especially in preclinical research and early clinical trials.

Similarly, RNA-based therapies have emerged as a promising avenue in drug development for cardiac diseases. Specifically, approaches like RNA interference and antisense oligonucleotides offer potential treatments in cardiovascular disease (Crooke et al, 2021). Many microRNAs or long noncoding-RNAs have been suggested to potentially enhance cardiac activity in acute MI, fibrosis, hypertrophy, or heart failure, among others (Lucas and Dimmeler, 2016; Lucas et al, 2018). A further successful concept of cardiovascular cell delivery using AAV was achieved using 2 proliferative miRNAs miR-500 and miR-199a to promote cardiomyocyte regeneration and recover cardiac function after MI in mice (Eulalio et al, 2012). Similarly, miR-1 expression demonstrated a potential novel therapeutic strategy to reverse pressure-induced cardiac hypertrophy and prevent maladaptive cardiac remodeling (Karakikes et al, 2013; Luo et al, 2018). Over the past decade, numerous oligonucleotide-based therapies have been developed using locked nucleic acid (LNA)-modified chemistries to modulate cardiac miRNAs. AntagomiRs and LNA antimiRs stand out as prominent examples in the category of miRNA inhibitors (Krützfeldt et al, 2005). Dysregulated miRNAs can contribute to the development or progression of cardiovascular pathological conditions by promoting harmful processes and antimiRs are therapeutic molecules designed to inhibit the function of specific miRNAs (Rupaimoole and Slack, 2017). LNA antimiRs are an advanced form, chemically modified for greater stability and stronger binding to target miRNAs (Samolovac and Hinkel, 2022). By blocking miRNA function, LNA antimiRs offer a promising approach to treating cardiovascular diseases by potentially reversing damage caused by abnormal miRNA activity (Samolovac and Hinkel, 2022). For instance, the therapeutic potential of miR-15 as a target for manipulating cardiac remodeling and function in ischemic injury has been validated (Hullinger et al, 2012). Additionally, therapeutic inhibition through LNA-based antimiRs targeting miR-208a has shown improvements in cardiac function and increased survival during heart failure (Montgomery et al, 2011). Another observation is that inhibiting the entire miR-34 family reduces cell death and fibrosis following MI (Bernardo et al, 2012; Yang et al, 2015). These advancements underscore the growing significance of LNA-modified chemistries and antimiRs in the development of oligonucleotide-based therapies, particularly in the context of cardiac miRNA modulation. Altogether, these RNA-based therapies hold promise because of their specificity and potential for personalized medicine. By targeting specific genes or molecular pathways implicated in cardiac diseases, they offer a tailored approach to treatment. Ongoing research aims to refine these therapies to enhance their efficacy, reduce off-target effects, and bring forth safer and more efficient treatments for multitude of

cardiac disorders (Cornu et al, 2017; Mann et al, 2002; Sasso et al, 2022; Dhakne et al, 2023).

In proof-of-concept studies, Nelson et al (2019) and Tabebordbar et al (2021) used AAV9 to deliver the CRISPR/Cas9 gene-editing system to young mice with a mutation in the gene coding for dystrophin deficiency in patients with Duchenne muscular dystrophy (DMD). Gene editing partially restored dystrophin protein expression in skeletal and cardiac muscle and improved skeletal muscle function. rAAV delivery of CRISPR/Cas9 or other enzymes to perform genome editing in mouse cardiomyocytes or hiPSC-CMs (as previously discussed) is a powerful tool in both gene therapy and generating new models. Novel Myo-AAV capsid has been used to achieve gene expression of CRISPR-based gene therapy treating dilated cardiomyopathy (Grosch et al, 2023). Thus, gene-editing systems elude any complication derived from vector size and can theoretically be applied to edit any gene across diverse genetic backgrounds. This technology could offer a suitable platform in treating cardiac diseases whose pharmacological spectrum fails in reducing arrhythmias.

X. Peptide-based treatment: new antiarrhythmic modality

Sudden cardiac death in children and young adults is a relatively rare but tragic event whose molecular pathophysiology is unknown and treatment is inadequate (Mazzanti et al, 2020; Moreno-Manuel et al, 2023).The identification of the interaction at the molecular level of the main sodium channel ($Na_V1.5$) with the strong inward rectifying potassium channel (Kir2.1) in the control of cardiac excitability and impulse conduction (Milstein et al, 2012) has opened a new paradigm for drug discovery and treatment of arrhythmias and SCD. Kir2.1 and $Na_V1.5$ form "channelosomes" that are anchored together at their eventual membrane microdomains by physically interacting via specific PDZ-binding domains in their respective -COOH terminal with scaffolding proteins like SAP97 and α1-syntrophin (Petitprez et al, 2011; Milstein et al, 2012; Gillet et al, 2015). Certain loss-of-function Kir2.1 mutations can also reduce $Na_V1.5$ function in Andersen-Tawil syndrome type 1, a rare but potentially lethal inheritable cardiac ion channel disease (Macías et al, 2022; Cruz et al, 2023; Moreno-Manuel et al, 2023). For example, arrhythmias associated with a trafficking deficient mutation (Δ314-315) in Kir2.1 alter both I_{K1} and I_{Na} (Macías et al, 2022). Similarly, another loss-of-function Kir2.1(C122Y) mutation that enables channel trafficking to the membrane but alters channel structure and biophysics also reduces both I_{K1} and I_{Na} leading to slow ventricular conduction and arrhythmias in ATS1 (Cruz et al, 2023). In many of these patients, the treatment provided by clinical guidelines is based on combinations of β-blockers and traditional class 1C AADs like flecainide. Such treatments are empirical, subject to trial and error and may even be proarrhythmic, likely because they impair sodium channel function (Kukla et al, 2014; Tristani-Firouzi and Etheridge, 2010; Mazzanti et al, 2020). Therefore, the development of advanced and targeted drug therapies based on small peptides that combine the elements necessary to transport and anchor Kir2.1 and $Na_V1.5$ to the cell membrane could have an impact in treating cardiac diseases related to ion channel remodeling. Patients with cardiomyopathy of DMD are at risk of developing life-threatening arrhythmias because of ion channel remodeling that reducing both I_{Na} and I_{K1} (Jimenez-Vazquez et al, 2022). Interestingly, transfecting just 1 component of the dystrophin protein complex (α1-syntrophin) restored channelosome function, increasing I_{Na} and I_{K1} densities in hemizygous iPSC-CMs from a patient with DMD (Jimenez-Vazquez et al, 2022). Thus, reimagining antiarrhythmic pharmacotherapy by developing bold and tailored peptidic solutions that restore channelosome dysfunction may have a powerful impact in treating a whole spectrum of cardiac arrhythmias, from the molecule to the bedside. The approach could offer improved efficacy and safety compared to conventional AADs and may target neurological disorders, cardiovascular diseases, or even certain types of cancer.

A new class of peptidic agents, known as peptibodies, is emerging. Peptibodies are chimeras generated as fusion proteins of the fragment crystallizable (Fc) domain of the human IgG with a bioactive "warhead" peptide (Cavaco et al, 2017; Shimamoto et al, 2012). Peptibodies combine the biologic/therapeutic activity of a given peptide, with the stability of monoclonal antibodies. They are stable and safe molecules that are developed as viable clinical therapeutics. For instance, the first peptibody in clinical use is romiplostim, which is approved for the treatment of immune thrombocytopenic purpura (Hubulashvili and Marzella, 2009). Romiplostim is composed of thrombopoietin mimetic peptides fused to the *C* terminus of the Fc region of human IgG (Wang et al, 2004; Nichol, 2006; Molineux and Newland, 2010; Molineux, 2011; McElroy et al, 2015). New studies have proposed the peptibody technology to selectively design peptides that bind to and modulate the function of specific ion channels involved in arrhythmogenesis (Ehrlich et al, 2008; Calvo et al, 2018; Heijman et al, 2018; Chidipi et al, 2022). Specially, in persistent AF, the acetylcholine-activated inward rectifier potassium current (I_{KACh}) is constitutively active, behaves as a background potassium inward rectifier channel, and can thus contribute to the initiation and maintenance of the arrhythmia (Dobrev et al, 2005; Heijman et al, 2018). Because I_{KACh} is mainly expressed in the atria and is largely absent from the working ventricular myocardium, it has been proposed that blocking I_{KACh} can be an atrial-selective rhythm control pharmacotherapy (Ehrlich et al, 2008; Calvo et al, 2018; Heijman et al, 2018). An I_{KACh} blocking peptibody (Fc-TP) was engineered as a fusion protein between the Fc fragment of the human IgG1 and a tertiapinQ peptidotoxin (TP) originally isolated from the venom of the European honey bee (Drici et al, 2000). Patch-clamping experiments showed that Fc-TP was approximately 300-fold more potent than TP alone, which could be due to an increased stabilization between the peptibody and I_{KACh}. Interestingly, the peptibody blocked carbachol-activated I_{KACh} in atrial tissue reducing inducibility of AF in aged mice while minimizing off-target effects (Chidipi et al, 2022), but it did not affect the potassium current in ventricular myocytes. Therefore, the advancement in designing peptibodies to target and modulate ion channels implicated in arrhythmias represents a step forward in the development of next-generation antiarrhythmic modalities with enhanced specificity and reduced adverse effects for a variety of diseases.

XI. Advancements in drug delivery

Traditional systemic drug administration can result in low drug concentrations in the heart and potential side effects in noncardiac tissues. Ensuring effective delivery of AADs to cardiac tissue remains a critical challenge (Nadimi et al, 2018). Recent advancements in drug delivery technologies are revolutionizing the landscape, offering new hope for improved therapeutic outcomes. Targeted drug delivery systems aim to overcome these limitations by selectively delivering medications to the affected cardiac tissue (Omidian et al, 2023). Development of nanoscale drug delivery systems offers unique advantages. Metal-based, lipid-based, and polymer-based nanoparticles represent ideal materials for use in cardiovascular therapeutics allowing enhanced drug stability, controlled release, and improved bioavailability (Qian et al, 2023). Nanoparticles can be engineered to allow encapsulated antiarrhythmic drugs navigate through the circulatory system and reach specific heart tissues (Nadimi et al, 2018; Wang et al, 2022; Yang

et al, 2022a). For example, a study demonstrated that liposomal amiodarone augments antiarrhythmic effects and reduces hemodynamic adverse effects in an ischemia/reperfusion rat model (Takahama et al, 2013). Many studies have reported the application of different nanodrug delivery systems charged with botulinum toxin, $CaCl_2$, L-glutamate, budesonide, carvedilol, and *N*-isopropyl acrylamide monomer (Wang et al, 2022) in the treatment of AF. Such precision minimizes off-target effects and enhances therapeutic efficacy. Moreover, surface modifications can enable nanoparticles to actively target areas such as inflammation or fibrosis within the heart (Omidian et al, 2023). Implantable devices, such as drug-eluting stents and patches, provide a localized and sustained release of antiarrhythmic medications (Fayzullin et al, 2021; Adhami et al, 2023). These devices can be strategically placed in or around the heart, ensuring continuous drug delivery to the affected area. For instance, drug-eluting stents have been designed to release medications directly into the coronary arteries, reducing the risk of reoccurrence of arrhythmias following interventions like catheter ablation (Adhami et al, 2023). The use of drug-eluting stents, among patients with MI and AF has increased over time (Vora et al, 2023a). In addition, innovations in electroresponsive drug delivery systems involve the incorporation of smart materials that respond to changes in the electrical environment of the heart. These systems can release drugs in response to specific cardiac conditions, offering a real time and on-demand therapeutic approach (Zhao et al, 2016). For example, several hydrogels with conductive properties have been designed to restore the electrical impulses to the heart, preventing further remodeling and ventricular dysfunction after MI (Tapeinos et al, 2022).

3D printing has emerged as a customized transformative technology allowing for the creation of intricately designed structures and personalized medications. 3D printing enables the fabrication of intricate drug formulations with precise control over drug-release kinetics. Antiarrhythmic medications can be embedded within biocompatible materials, such as polymers, in a controlled and layered fashion (Vaz and Kumar, 2021). This design allows for sustained and targeted drug release, ensuring optimal therapeutic concentrations in the affected cardiac tissue. For example, the production of 3D-printed preparations of high-quality filaments containing amiodarone and dronedarone has been described in the literature (Matijašić et al, 2019; Roulon et al, 2021). A recent study also prepared propranolol hydrochloride gummy chewable tablets tailored for children by semisolid extrusion 3D printing technology to meet personalized medicine needs in pediatrics (Zhu et al, 2022a). 3D printing also facilitates the incorporation of multiple medications into a single, complex structure. This capability is particularly valuable in antiarrhythmic therapy, where combination drug regimens may be more effective (Lu et al, 2023). The versatility of 3D printing extends to the creation of implantable devices specifically designed for antiarrhythmic drug delivery. Implantable patches, stents, or microstructures can be customized to fit the unique anatomy of the heart, offering localized and sustained release of medications (Domsta and Seidlitz, 2021; Lu et al, 2023). These devices can be strategically placed to deliver drugs directly to the affected areas, improving the precision of treatment and reducing the risk of adverse effects. There are challenges related to 3D printing, which include ensuring the biocompatibility of printed materials, refining printing techniques for pharmaceutical applications, and navigating regulatory pathways. While these new drug delivery technologies hold immense promise, several challenges must be addressed to bring this technology to widespread clinical use. Optimizing biocompatibility, long-term stability, and scalability of these technologies remains a focus of ongoing research.

XII. Concluding remarks

Current antiarrhythmic therapies have limited efficacy and are frequently associated with adverse effects. Alarmingly, while the number of new therapies under investigation in other fields is growing exponentially, the number of novel antiarrhythmic targets and agents in the development pipeline has decreased substantially during the last few decades because of conceptual, regulatory, and financial considerations (Saljic et al, 2023). Catheter ablation significantly contributes to the management AF, particularly in patients who are refractory or intolerant to antiarrhythmic drugs (Chugh et al, 2014; Parameswaran et al, 2021). The CABANA (Catheter Ablation vs Anti-arrhythmic Drug Therapy for Atrial Fibrillation; NCT00911508) trial's findings are particularly notable, demonstrating the effectiveness of catheter ablation in reducing the recurrence of AF and symptomatic episodes compared with drug therapy over a 5-year follow-up period (Poole et al, 2020). It is crucial to consider not only the physical outcomes but also the impact of interventions such as catheter ablation on mental health in patients with AF. ANZCTR trial data (ACTRN12618000062224) suggest that catheter ablation may lead to greater improvements in markers of psychological distress, specifically symptoms of anxiety and depression, compared to medical therapy alone in individuals with symptomatic AF (Al-Kaisey et al, 2023). Nevertheless, it is not a universally applicable solution, and the proposition of subjecting over 46 million patients with AF to 1 or more ablation procedures would be exceedingly unreasonable. Consequently, arrhythmia treatment poses a significant challenge in the realm of cardiovascular health, prompting the need for alternative approaches. This has a point of complexity given that the heart is difficult to access, donors are limited, and data from rodent models are hard to extrapolate because the cardiac electrophysiological characteristics of small animals are significantly different from humans. This forces the use of large animals such as sheep, dogs, or pigs that have major cost and ethical limitations. Yet, disparities between animals and humans can confound results and likely contribute to the failure of promising therapeutics when advancing to later stage clinical trials. Fortunately, new technologies and computational models are now available to solve these problems. Last year, the FDA Modernization Act 2.0 paved the way for alternative methods to bolster the preclinical data pipeline, aiming to reduce the dependence on animal models that have frequently resulted in therapeutic dead ends. The FDA Modernization Act 2.0 reinforced the transitioning beyond animal models with human cells, organoids, and AI/ML-based approaches (Zushin et al, 2024). Despite all advantages that hiPSC-CMs can hold to the discovery and development of antiarrhythmic drugs, it is necessary to keep in mind all current technical, economic, and time limitations that need to be addressed, mainly in terms of electrophysiology. The electrophysiological maturation state of hiPSC-CMs can determine drugs responsiveness (da Rocha et al, 2017). As discussed throughout this review, the advent of new and emerging tools that combine biological systems like stem cells with cell profiling platforms (multiomics), new technologies for data processing, computational modeling, and functional HTS techniques have made drug discovery and design much more sophisticated and technology driven. This will improve the quality, safety, and efficacy of future antiarrhythmic therapies by enhancing precision and personalized medicine. In this review, we especially highlighted the promising therapeutic potential of alternatives like gene therapy and the use of peptide-based therapies in the treatment of arrhythmias. It is important to note that both technologies are dynamic and subject to ongoing research and development. As we previously highlighted, gene therapy has the potential to treat arrhythmias by correcting or compensating for the genetic defects that cause abnormal heart rhythms,

potentially providing a long-term solution. However, limitations include difficulties in delivering genes safely into heart tissue and ensuring precise and long-lasting effects without unintended consequences. The other alternative, peptibodies, designed and engineered to target specific proteins or receptors, offer several potential advantages in treating arrhythmias like reduced side effects, modularity and customization, and biological compatibility (Chidipi et al, 2022). While peptibodies hold promise in the treatment of various conditions, including arrhythmias, there are certain limitations and challenges associated with their use. Those are delivery and stability, immunogenicity, short half-life, development costs, lack of long-term safety, regulatory approval hurdles, and patient variability. However, ongoing research and technological advancements may address some of these limitations over time. In summary, the use of hiPSCs, together with new emerging technologies and computational integration, offers the unprecedented possibility of improving the discovery of arrhythmia targets, biomarkers, and drugs. New alternatives such as gene therapy and peptide-based therapy open a new path for the development of a new and promising next-generation antiarrhythmic therapy with great clinical translation potential.

Abbreviations

AAD, antiarrhythmic drugs; AF, atrial fibrillation; AI, artificial intelligence; AP, action potential; AVN, atrioventricular node; CiPA, comprehensive in vitro proarrhythmia assay; CPVT, catecholaminergic polymorphic ventricular tachycardia; DMD, Duchenne muscular dystrophy; ECM, extracellular matrix; Fc, fragment crystallizable; HR, heart rate; HTS, high-throughput screening; hERG, human ether-a-go-go-related gene; hiPSC, human-induced pluripotent stem cell (hiPSC); hiPSC-CM, human-induced pluripotent stem cell–derived cardiomyocytes; I_{KACh}, acetylcholine-activated inward rectifier potassium current; I_{K1}, inward rectifying potassium current; I_{Kr}, delayed rectifier potassium current; I_{Na}, sodium current; LNA, locked nucleic acid; LQTS, long QT syndrome; MI, myocardial infarction; OoC, organ-on-a-chip; QSAR, quantitative structure-activity relationship; rAAV, recombinant adeno-associated virus; RyR, ryanodine receptor; SCD, sudden cardiac death; SNP, single-nucleotide polymorphism; TdP, Torsade de Pointes; TP, TertiapinQ peptidotoxin; VUS, variants of uncertain significance.

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Conflicts of interest

The authors declare no conflict of interest.

Data availability

The authors declare that all data supporting the findings of this study are contained within the paper.

Author contributions

Performed data analysis: Mondéjar-Parreño.

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