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SimCardioTest - Simulation of Cardiac Devices & Drugs for in-silico Testing and Certification

Technical Report

D 5.3: REPORT ON THE IN-SILICO TRIAL OF DRUG EFFICACY & CARDIOTOXICITY

Work Package 5 (WP5)
IN-SILICO TRIALS & DATA SCIENCE

Task Lead: UPV, Spain
WP Lead: INRIA, France

DELIVERABLE INFORMATION

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Deliverable title	Report on the in-silico trial of drug efficacy & cardiotoxicity
Description	In-silico trial tool to determine drug concentrations assuring TdP-related safety in different groups of patients. Example with Dofetilide
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Executive summary

This report constitutes the SimCardioTest WP5 deliverable D5.3 due in December 2023 (M36) related to Use Case 3.
Here, we describe the protocol to conduct an in-silico trial integrated into a cloud-based platform. The software was adapted to use case 3, in which we use computational modelling and simulation of cardiac electrophysiology and mechanics to assess drug safety and efficacy. The tool was developed to evaluate the TdP-risk of molecules at different concentrations, a context of use in which model credibility has been previously assessed.

Acronyms

Table 1: List of Acronyms

Acronym	Meaning
AP	Action Potential
EFTPC	Effective free therapeutic plasma concentration
EM	Electromechanics / -ical
EP	Electrophysiology/ -ical
PK	Pharmacokinetics
SVM	Support Vector Machine
TdP	Torsade de Pointes

Preamble

NOTE: This section is identical for deliverables D5.1, D5.2 and D5.3.

In-silico trials based on computational models and simulation (CM&S) represent a revolutionary approach in the field of medical research and development. In-silico trials can be used in the development and regulatory phases of a new therapy, with the goal of reducing, refining and sometimes partially replacing clinical trials by the mean of individualized simulations of specific patients and sub-populations, and the synthetic effect on them of the targeted treatment [1,2]. In-silico trials are gaining visibility and increasing acceptance by regulatory authorities ([3,4], with a focus on cardiology), and dedicated organizations have been created, such as the Virtual Physiological Human institute (https://www.vph-institute.org), which promotes the implementation of CM&S to optimize clinical trials, and the Avicenna alliance (https://avicenna-alliance.com), founded by the European commission to develop the research roadmap for in-silico medicine, put it into policy and ensure the development of a regulated in-silico market. However, the widespread adoption and acceptance of in-silico trials requires a standardized framework to ensure consistency, reliability, and regulatory compliance. Recognizing this need, efforts are underway to establish Good Simulation Practice (GSP) guidelines. Scheduled for publication in 2024, the book "Toward Good Simulation Practice" (https://link.springer.com/book/9783031482830) is the result of the joint work of 138 In-silico Trials experts working in academia, the medical industry, regulatory bodies, hospitals and consultancies. This initiative aims to create a unified approach that will enhance the credibility and acceptance of in-silico trials across the scientific and regulatory communities.

To complement the standardization effort, there is a clear need for the community to agree on protocol and reporting standards. Inspired by the well-established Good Clinical Practice (GCP) framework, a protocol has been created within SimCardioTest to drive researchers through an efficient and effective deployment of in-silico trials. Each Use Case implemented in WP2-3-4 performed a specific in-silico trial based on the proposed in-silico protocol, and carried out via the secure standardized cloud-based platform developed in SimcardioTest. Deliverables D5.1-2-3 reports the details and results of the in-silico trials for Use Case 1, 2, and 3 respectively.

[1] Pappalardo, F., Russo, G., Tshinanu, F. M., & Viceconti, M. (2019). In silico clinical trials: concepts and early adoptions. *Briefings in bioinformatics*, *20*(5), 1699-1708.

[2] Viceconti, M., Juárez, M. A., Curreli, C., Pennisi, M., Russo, G., & Pappalardo, F. (2019). Credibility of in silico trial technologies—a theoretical framing. *IEEE journal of biomedical and health informatics*, *24*(1), 4-13.

[3] Miller, C., Konduri, P., Bridio, S., Luraghi, G., Terreros, N. A., Boodt, N., ... & Hoekstra, A. (2023). In silico thrombectomy trials for acute ischemic stroke. *Computer Methods and Programs in Biomedicine*, *228*, 107244.

[4] Rodero, C., Baptiste, T. M., Barrows, R. K., Keramati, H., Sillett, C. P., Strocchi, M., ... & Niederer, S. A. (2023). A systematic review of cardiac in-silico clinical trials. *Progress in Biomedical Engineering*.

In-silico protocol development process

The in-silico trials realized for each UC within SimCardioTest whose results are reported in deliverables D5.1, D5.2 and D5.3, have been carried out following a common in-silico protocol, developed within the project. The objective was to have a standardized template allowing to organize and perform all relevant steps to execute, analyse and assess an in-silico trial. This process, together with the realization of the subsequent in-silico trials, required coordination across all UC's partners, IST, and Inria. Here below we list the different phases and main meetings we organized during the period M24-M36.

Table 1. Main phases in the development of the standardized in-silico trial protocol and in-silico trial set-up.

Date	Participants	
21/06/2022	UPV, SIMULA, EXC, IST, Inria	Preliminary discussion based on UC3 and initial protocol draft
20/07/2022	UPV, SIMULA, EXC, IST, Inria	Follow-up discussion and continuous improvement of the first draft.
07/11/2022	Inria	Communication of the template for in-silico protocol to all UC's partners.
18/11/2022	UBX, UPF, UPV, IST, Inria	WP5 meeting on in-silico trial protocol design.
20-21/03/2023	All UC's partners, IST, Inria	One day hands-on on in-silico trials during the annual SimCardioTest GA to specialize an in-silico protocol for a selected QoI and at least one CoU per UC.
March to November 2023	All UC's partners, IST, Inria	Regular monthly UC-specific meetings for updates on the advancements on the in-silico trials, refinement of the protocol, and of the UCs' dedicated interfaces.
07/11/2023	All SCT partners	Half-day workshop on in-silico trials.
November to December 2023	All UC's partners, IST, Inria	The protocols are finalized, in-silico trials are run offline. The integration of the latest version of the codes advanced in parallel. Regular monthly UC-specific meetings are organized for coordination.

1 Introduction

1.1 BACKGROUND INFORMATION

An important and limiting phase in drug development is to pass the regulatory evaluation of new compounds. Pharmaceutical companies need to provide solid evidence of the efficacy and safety of the molecules, which requires a large amount of in vitro and in-vivo tests, elevating development times and production costs. Safety pharmacology is a key step during the assessment of drug candidates to detect adverse effects, including tests evaluating potential proarrhythmic effects. The objective is to identify and discard molecules generating Torsades de Pointes (TdP), a life-threatening ventricular arrhythmia.

Mathematical models describing cardiac electrophysiology have progressed over the years, and it is possible to exploit simulation tools for safety pharmacology studies. However, model credibility is crucial to provide reliable evidence accepted by regulatory bodies, so verification and validation tasks were performed previously. All activities were defined in a specific context of use related to the preclinical stage of drugs, where model predictions intend to complement in-vitro and animal studies, and help design future clinical trials.

Considering the TdP risk induced by drugs and its dose dependence, we posed the following question of interest: “What is the maximum concentration/dose regimen of a drug to assure TdP-related safety in a population of healthy subjects?”. Based on this context of use focused on drug safety assessment, we defined a modeling and simulation protocol to conduct an in-silico trial considering different characteristics of the populations. The present in-silico trial aims to provide a complementary approach that guides clinical decisions by identifying drug concentration thresholds with potential TdP-risk. Dose adjustment can prevent malignant proarrhythmic events in patients subjected to a pharmacological treatment and improve clinical trial protocols.

Risks and Benefits

The main known benefits of including in-silico trials in the regulatory evaluation process are the reduction, refinement, and partial substitution of current in-vivo studies (animal and human). Consequently,

- Virtual models are adapted to human characteristics, which avoids species differences.
- Unlike a clinical trial, the size of the virtual cohort group can be easily increased.
- The results obtained with a virtual set of patients complement clinical trial results, which allows the reduction of enrolled patients.
- in-silico trials allow a better evaluation of TdP-risk in subpopulations (male vs female), by identifying the dose threshold per subpopulation.
- Possibility to explore conditions not achievable in clinical trials, such as a higher drug dose.
- in-silico trial results contribute to refining the inclusion criteria of a clinical trial and can provide additional outcomes.

The potential risks are model prediction inaccuracies:

- False negatives and/or false positives can be predicted due to uncontrollable factors. The risk is more considerable when the dose threshold is estimated over the actual value, and patients are exposed to drug-induced proarrhythmic events.

- Scarce validation data for some patients (e.g., women) limits the prediction power in those particular subgroups.

1.2 OBJECTIVES

The aim of the present in-silico trial is to show the capabilities of cardiac electrophysiology numerical models that have been previously verified and validated. Developed models are designed to be deployed in the frame of drug development and to focus on assessing TdP-risk, in line with the context of use. During preclinical phases, computational simulation techniques are intended to complement experimental results and improve the design of future clinical trials through population stratification. Although the population is limited to healthy adults (males and females), the methodology here presented could be extended to children and pathologies.
According to the defined context of use, the in-silico trial should fulfil the following objectives:

Primary objective
To inform about safe conditions before a real clinical trial by classifying pharmacological compounds according to the risk to induce TdP.

Secondary objectives
To find critical dose/drug concentration that assures TdP-safe conditions.
To achieve a better stratification of the population to adjust drug doses to subgroup characteristics.
To guide clinical decisions and improve the design of new clinical trials.

Description of the case of study
The in-silico trial we designed to assess the TdP risk of compounds was analysed in a particular case to ensure the accomplishment of the objectives. We selected the drug Dofetilide to show the simulations pipeline and the potential of in-silico trial results.

Dofetilide is a class III antiarrhythmic agent that can cause severe ventricular arrhythmias, mainly TdP. Regulatory agencies approved it because it was effective in terminating supraventricular arrhythmias, such as atrial fibrillation and flutter, and restore normal sinus rhythm (Falk et al., 1997). However, serious safety concerns arose later. It was found that Dofetilide had a high risk of inducing TdP and that the incidence increased with plasma concentration (Bianconi et al., 2000). Given that several factors can affect drug plasma concentration, rigorous dosing guidelines are needed to regulate the administration of Dofetilide and minimize its proarrhythmic risk. Special caution should be taken when used in women because Dofetilide was associated with a greater risk of TdP in female patients than in males, as with other drugs that cause TdP (Accord Healthcare, 2022).

2 Study Design

2.1 VIRTUAL POPULATION DEFINITION

The virtual population for TdP-risk classification consisted of human cardiomyocytes with EP activity. A secondary three-dimensional electromechanical simulation in a wedge of ventricular tissue complemented the cellular one.

Interindividual variability is defined by specific demographic, genetic, or environmental characteristics (known as covariates) of the pharmacokinetic (PK) and the EP models. Covariates that affect PK are molecule dependent. For instance, the variables sex (male vs female), weight (in kg), and renal status (expressed as glomerular filtration rate, GFR) were specific for Dofetilide. While sex is a categorical variable, weight was limited to the standards for each sex and related to the average height (Table 2). Renal status was turned into a categorical variable called renal function with two options: normal and impaired function associated with GFR values of 90 and 60 mL/min/1.73 m^2, respectively. Despite the existing variability among patients, an heterogenous population of PK was not implemented for this in-silico trial. Each virtual population had specific fixed PK covariates instead, which allowed the comparison between generated groups. Therefore, the interindividual variability within the population subjected to the in-silico trial was generated basically from the variability in EP properties.

Table 2: Standard reference values of height and weight in adults

	Average Height	Weight range
Male	1.79 cm	60 kg - 80 kg
Female	1.66 cm	50 kg - 70 kg

From the electrophysiological point of view, we can distinguish between male and female myocyte models, which have different parametrization in some cellular proteins. The biological uncertainty related to these parameters was considered by applying a normal distribution of mRNA channel expression based on the means and standard deviations of the experimentally reported gene expression levels for each sex. The EP changes due to different hormonal levels were also reproduced in the population.

The ion channels involved in sex differentiation were the rapid and the slow delayed rectifier potassium current (I_{Kr} and I_{Ks}), the transient outward potassium current (I_{to}), the inward rectifier potassium current (I_{K1}), the sodium current (I_{Na}), the L-type calcium current (I_{CaL}), the sarcolemmal calcium pump current (I_{pCa}), the sodium-potassium ATPase current (I_{NaK}), the sodium-calcium exchange current (I_{NCX}), the background potassium current (I_{Kb}), and the calcium uptake via SERCA pump (J_{up}); the maximum calmodulin concentration was also different. A second set of modifications to reproduce hormonal levels was applied to I_{Kr}, I_{Ks}, and I_{CaL}. The initial generated populations for each sex were 50,000 different cells obtained from random combinations of the 12 parameters mentioned above. The next step was a calibration process to exclude models with EP properties out of the physiological range, and final subsets of 300 cells from the accepted models were selected (Llopis-Lorente et al., 2023).

For the three-dimensional trial, we selected one representative model from the population to quantify the electromechanical effects of Dofetilide. The ventricular wedge consisted of a cardiac slab of dimensions 6x2x2 mm^3. The properties of the cardiac tissue were generic and independent of the patient, and only the genotype of the selected cellular model determined the results.

2.2 INTERVENTION DEFINITION

The intervention is determined by the variables dose, administration interval, and release form. Duration is not a parameter of the in-silico trial because only one short time window is analysed, as explained below. There are multiple possible combinations to define a Dofetilide intervention. One example used is a dose of 0.5 mg, with an interval of 12 h and an immediate release form.

The in-silico trial was designed to treat all the population with the same dosage scheme to find the critical one. Indeed, the same range of plasma concentrations was tested on both subpopulations (male and female), and when possible, they were translated to dosage. We started with an effective dose regimen converted to plasma concentration with pharmacokinetic models, and the value was reduced or increased until finding the limit value that assured safe TdP conditions.

Pharmacokinetic (PK) was specific for each molecule and the PK profile of Dofetilide is defined in Table 3. Despite obtaining the time course of drug plasma concentration, only the EP activity of the highest peak concentration was evaluated, given that larger concentrations are the most critical for TdP risk.

Table 3: Dofetilide PK data

Oral bioavailability	> 90%
Elimination half-life	10 hours
Volume of distribution	3 L/kg
Tmax	2.5 hours
Plasma protein binding	60-70%

The molecular EP target profile of drugs was considered multichannel and seven cardiac ionic currents (I_{Na}, I_{NaL}, I_{Kr}, I_{to}, I_{CaL}, I_{K1}, and I_{Ks}) were candidates for modulation. The drug effect on each channel was defined by two parameters (IC_{50} and Hill coefficient) and obtained from in-vitro channel block experiments. Dofetilide mainly blocks I_{Kr}, but we performed an extensive review in the scientific literature, including the search in public databases (e.g. DrugBank), to gather channel block data and we obtained information for six currents. The values used for the present in-silico trial case are shown in Table 4 and have been previously validated for this particular context of use. They correspond to the median of all existing values, with the exception of I_{Kr}, which value was readjusted. However, channel block values could be modified with updated research data.
The model evaluates drug effects in a particular time instant delimited by the duration of one cardiac beat (1 second) after model variables were stabilized. This explains why intervention duration did not apply in the in-silico trial we designed.

Table 4: Channel block data for Dofetilide

	I_{Kr}	I_{Na}	I_{NaL}	I_{CaL}	I_{Ks}	I_{K1}	I_{to}
IC_{50} (nM)	15	147900	753160	26700	100000	-	18.8
h	1	1	1	1	1	-	0.8

2.3 IN-SILICO TRIAL DESIGN

We implemented a four-module in-silico trial: selection of model inputs, simulation, output visualization, and evaluation. The simulation part consists of 3 independent sections, which are three modelling approaches with their inputs and outputs but with some interactions according to the workflow illustrated in Figure 1. Briefly:

1) PK section is the first part of the in-silico trial, requiring patient features and drug dosage selection as inputs. It provides plasma concentration over time to be used as input in the EP model. It requires of the following transformation to free plasma concentration (FPC):

$$FPC = \frac{10^6 \cdot (1 - bound\ fraction) \cdot plasma\ concentration\ (mg/L)}{molecular\ weight\ (g/mol)}$$

2) Cellular EP section computes the electrophysiological activity in a myocyte (action potential) based on individual cell genotypes and drug blockage effects. Generated outputs provide the biomarkers used for the TdP-risk evaluation.

3) 3D EM section computes the electromechanical activity in myocardial tissue. It is composed of the same myocytes used in EP section and provides additional outputs related to mechanical properties.

The main results of the in-silico trial are given in the evaluation module, which consists of a machine learning classification approach. As it is implemented so far, the classifier only requires EP biomarkers to determine TdP-risk. Additional outputs such as mechanics and p-ECG are shown as complementary results.

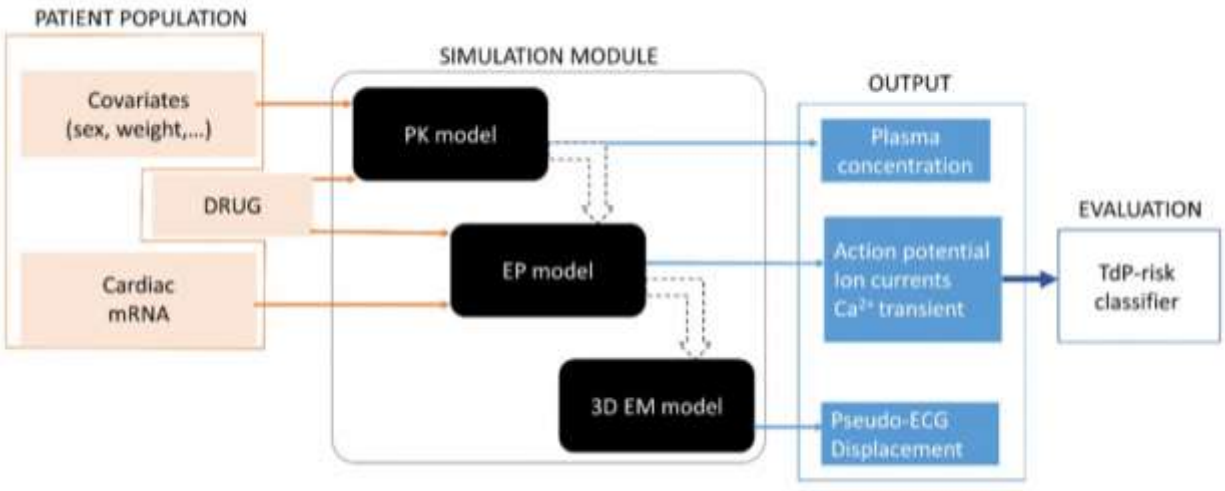


***Figure 1**: Schematic view of the in-silico trial workflow*

Virtual patients' generation

The in-silico trial was designed to perform the tests always on the same virtual cellular population (300 males and 300 females) defined with EP parameters. One advantage of in-silico simulations is that different conditions can be tested once and again on the same individuals without needing to create multiple arms. That said, the entire set of cellular models were subjected to the same pharmacological interventions, allowing the comparison between different scenarios. It is to be noted that the EP properties of the population of cardiomyocytes in absence of pharmacological treatment (control population) were considered normal after having excluded cells with non-physiological activity during population generation. Therefore, from a cardiac point of view, all the individuals in the populations were healthy patients. The hormonal levels selected for each subpopulation were defined in the control population and were maintained during the different trials: male population was simulated with a dihydrotestosterone concentration of 35 nM, reflecting the normal high ranges in post-pubescent pre-senescent males, and female population was simulated during the early follicular phase since susceptibility to arrhythmias increases during this stage.

The virtual trials showed in this report as an example of the in-silico trial tool, were particularized to Dofetilide in a group of patients with specific selected characteristics. All patient covariates different than sex were fixed during the in-silico trial to perform patient stratification due to their gender. This way, weight was set to 70 kg and renal function to a healthy state with GFR equal to 90 mL/min/1.73 m^2).

Outputs

The in-silico trial recorded the time course of different types of signals on each simulation module:
- PK simulations led to the time course of drug plasma concentration. It extended over 350 hours to reach the steady state.
- Action potential (AP) and calcium transient were captured among the large number of EP variables at the cellular level, for being the most representative. Although 500 beats were simulated to reach the steady state, only the last beat was recorded and postprocessed.
- 3D simulations results were the p-ECG and wedge displacement, also from last beat after reaching a stable state.

3 Evaluation Plan

3.1 EVALUATION METRICS

The torsadogenic risk was evaluated with a classification tool that used several in-silico biomarkers as inputs. Simulations led to a variety of EP outputs from which several indices were computed in a post-processing phase to facilitate the interpretation of results. The following cellular biomarkers were considered in the present in-silico trial:

APD_{90}: the duration of AP at 90% of repolarization,
Tri_{90_50}: triangulation 90-50, defined as APD_{90}-APD_{50},
Tri_{90_30}: triangulation 90-30, defined as APD_{90}-APD_{30},
qNet: net charge throughout the AP,
SystCa: systolic diastolic intracellular calcium concentration,

DiastCa: diastolic intracellular calcium concentration,
$CaTD_{90}$: calcium transient duration at 90% recovery,
$CaTD_{50}$: calcium transient duration at 90% recovery,
EMw_Ca: electromechanical window with calcium, defined as $CaTD_{90}$-APD_{90}

Most biomarkers calculated in this in in-silico trial were EP properties that informed about the cellular changes provoked by drugs. The basic approach to assess drug effects was the comparison of the indices before and after each intervention, and figures helped illustrate the differences. Population biomarkers were reported with the means and standard deviations.
Some biomarkers, such as APD_{90}, can be informative regarding the potential risk of TdP under certain conditions, but one single index does not have enough predictive power to discern between safe and risky scenarios. Therefore, all the data generated in the simulations was explored with machine learning techniques to create a prediction tool.

Additional biomarkers were obtained from the 3D simulations, complementing classification results:
- CV: conduction velocity, as a measure of electrical propagation in tissue.
- FS: fractional shortening, representing size changes in the wedge during systole.
- QT interval, the interval between waves Q and T measured on the pECG.

3.2 STATISTICAL DESIGN AND DATA ANALYSIS

The in-silico trial includes a data analysis part to answer the question of interest. It consists of a decision support model to classify drugs according to the TdP risk. We employed a classifier that was initially developed to assign a risk category to a set of medicines, but here, it was adapted to identify the safe dose of the evaluated drug.

Figure 2 shows the iterative evaluation process, which involves cellular EP simulation and classification techniques. The PK simulation is a prior step outside the loop and the EM simulation is a complementary activity to current TdP-risk evaluation. This flowchart is identical when assessing drug effects in single cells or in a population of models, but the classification method differs. The evaluation in single generic cells is performed with decision trees, and support vector machine (SVM) techniques are used to classify populations.

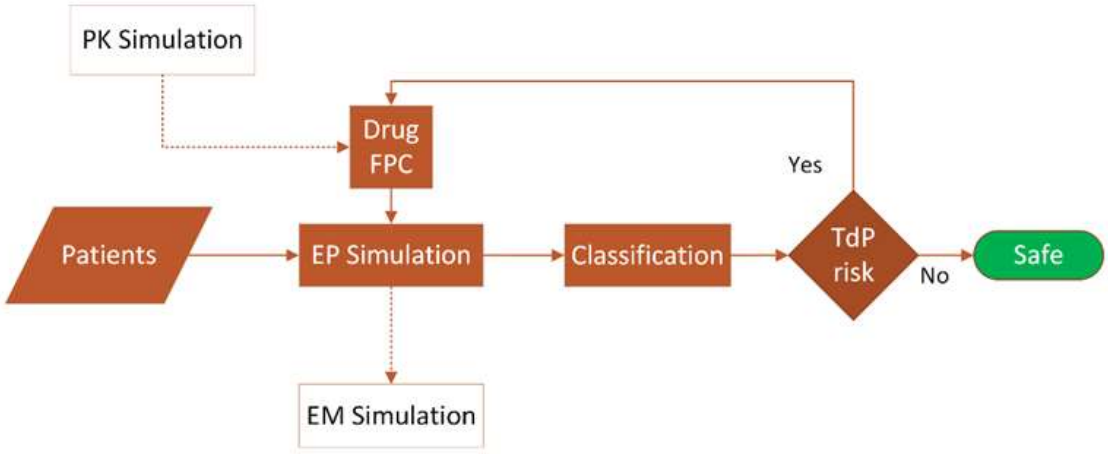


Figure 2: *TdP-risk evaluation flowchart*

Decision Trees

We developed a TdP-risk classifier for drug action on generic single cells (Llopis-Lorente et al., 2020) that combined three indices (T_x, T_{qNet}, and T_{triang}) based on APD, q_{Net}, and the predisposition to early-afterdepolarizations, respectively. It was created by applying a 9-fold cross-validation approach on a dataset of 109 compounds, which resulted in nine classification trees. The output of each decision tree was a classification of drugs in the safe or unsafe group, and the final label was obtained by majority voting technique of all trees.

In the in-silico trial, we used these decision trees on single generic cells to obtain a fast approximate result before testing on populations, firstly to know the category of the molecule with a selected dose regimen, and secondly to find the maximum safe drug concentration. The whole process is an iterative process that requires to increase or decrease the FPC manually.

Ternary Support Vector Machine

We developed a second classifier that assesses drug-induced TdP-risk on populations of cardiomyocytes (Llopis-Lorente et al., 2022). SVM method is applied to assign one of the three possible labels (high, intermediate, and low risk) to each individual, and then the percentage of models in each category is processed with two logistic regressions to yield the TdP-risk class of the drug on the population.

A total of 27 biomarkers are used as inputs for the ternary classifier, 9 cellular biomarkers (APD_{90}, Tri_{90_50}, Tri_{90_30}, qNet, SystCa, DiastCa, $CaTD_{90}$, $CaTD_{50}$, and EMw_Ca,) obtained from simulations at 3 different concentrations: 1 time, 5 times, and 10 times the concentration under study. The classifier was trained with 12 CiPA drugs selected from the 3 categories in equal proportion (Azimilide, Bepridil, Sotalol, Vandetanib, Chlorpromazine, Cisapride, Terfenadine, Ondansetron, Diltiazem, Mexiletine, Metoprolol and Loratadine). Dofetilide was excluded from this training group intentionally to be evaluated during the in-silico trial.

We defined a TdP-score calculated from the regression results and normalized to the range [-1 1], where -1 denotes that all the cells of the population have low TdP-risk and 1 that all the models are predicted as high risk.

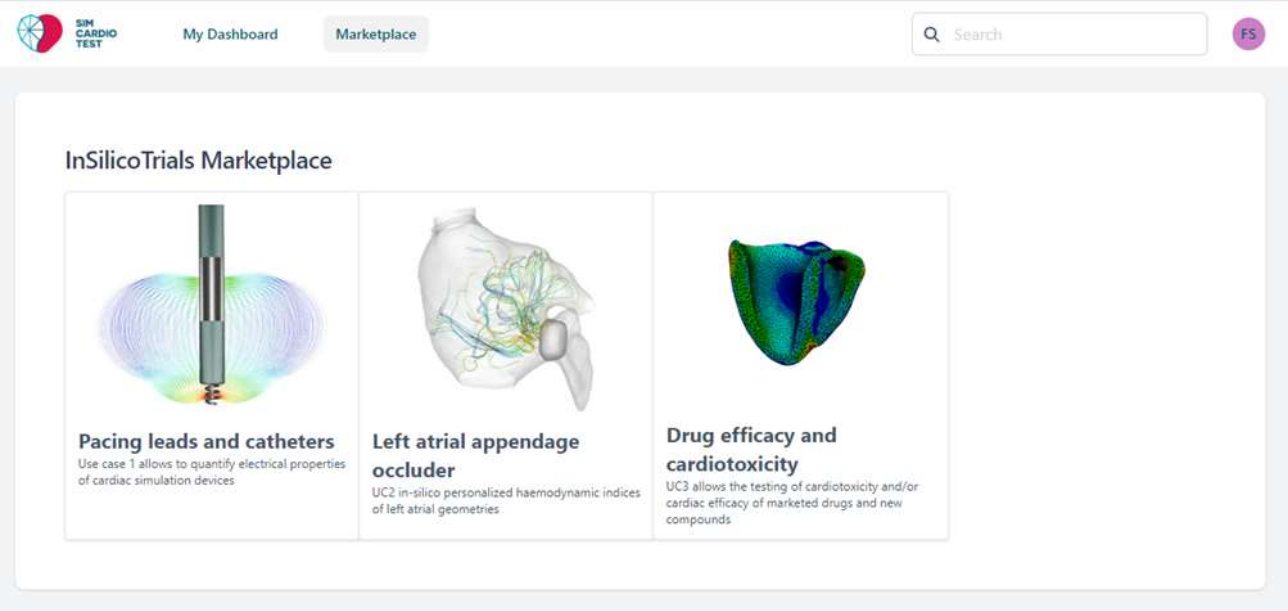
For the evaluation, an initial test applying the same dose to the different subgroups determines the need to increase or reduce drug concentration until finding the safe limit, i.e. when the label risk turns to “Low”. We assumed that this classifier performed similarly to the decision trees although it allows to refine the results to subpopulations.

4 In-silico trial Simulation Plan

The in-silico trial simulation will be simulated in the IST cloud-platform created for that purpose (https://sct.insilicotrials.com/). The platform combines user-friendly simulation set-up with cloud-based simulation and easily accessible results and evaluation. Here, we show step by step how an in-silico trial on the platform is conducted, using Dofetilide as an example. Additionally, the usage of the platform is shown in the associated video (Demonstration UC3 In Silico Trial).

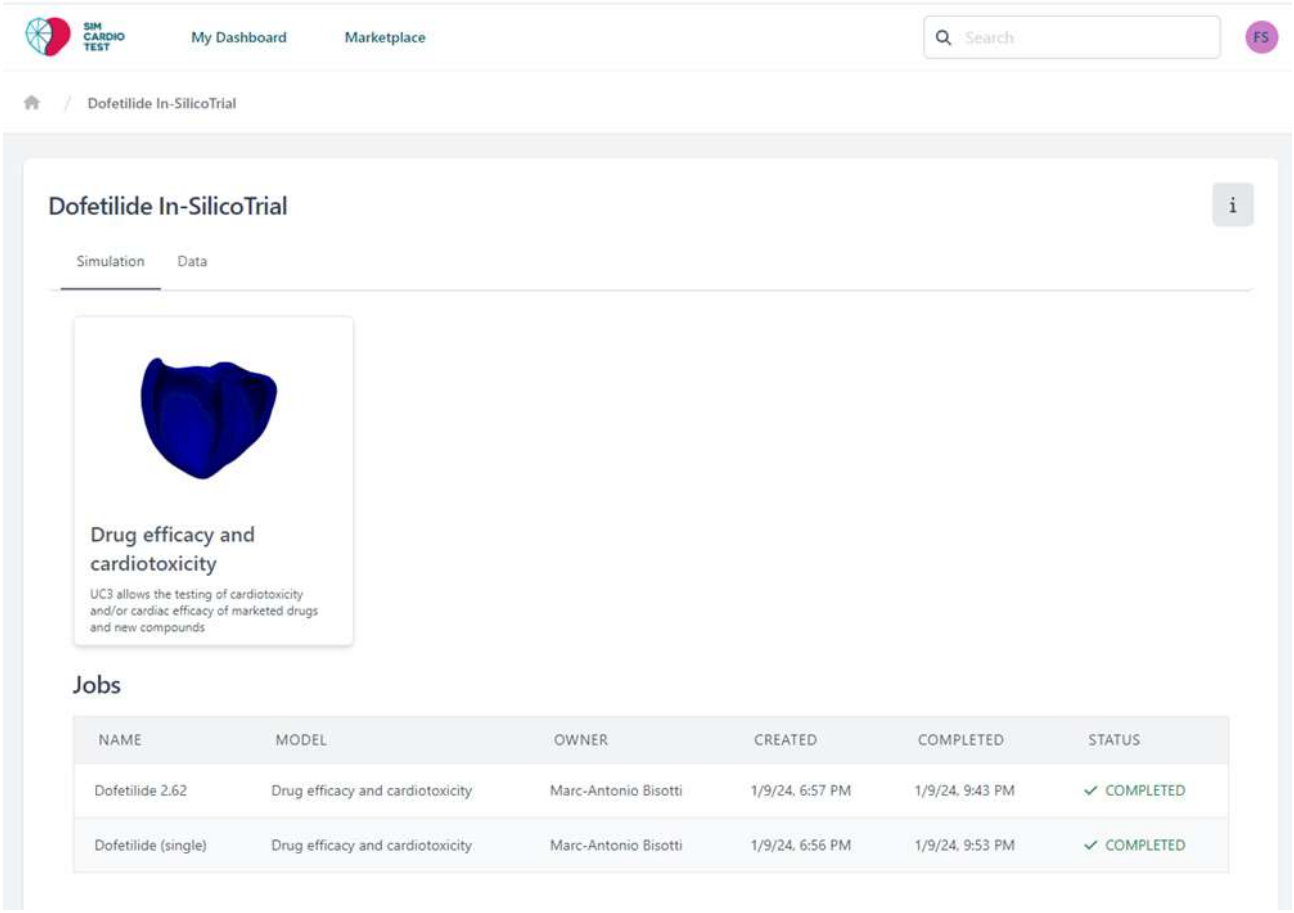
Platform home page

On the platform homepage, the user workflows corresponding to each of the three Use Cases are made available.



By going to their personal dashboard, a user can generate studies and simulations. The dashboard provides an overview of studies available.

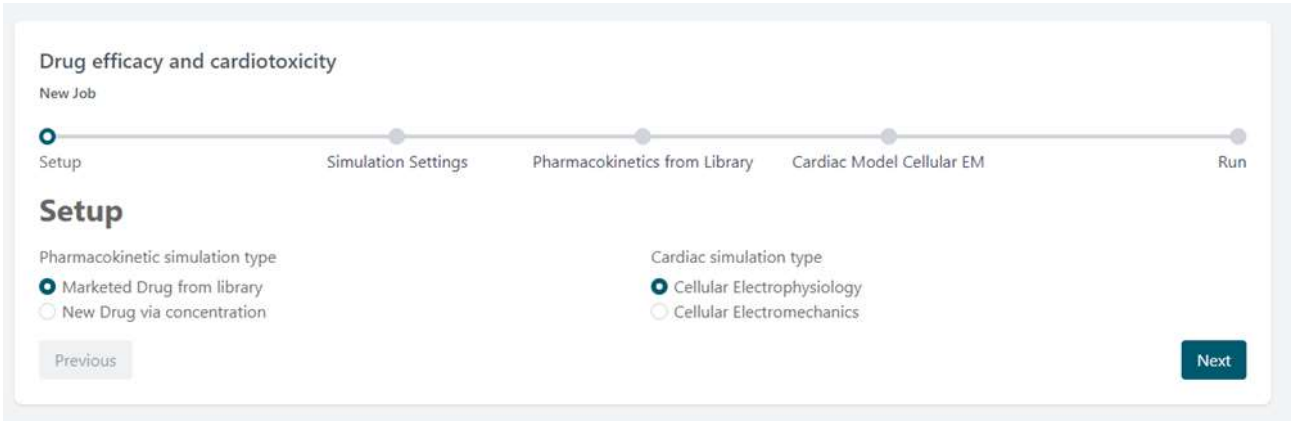
In the In-Silico Trial space, all simulations associated with this study are summarized.



By creating a new job, the user can enter the user-friendly environment that will guide them to create a new simulation.

Step 1. Request user inputs

In the simulations set-up environment, the user is guided step-by-step through the settings required by the models. The user can choose between the pharmacokinetic, electrophysiological and electromechanical models developed in this use case.



The pharmacokinetic model, for example, is accessed by an interactive page in which the user can first select a drug, and is then given a choice of formulations and asked to provide covariates needed for an accurate simulation of drug concentration, as well as dosing schedule.
Please note that for the purpose of this In Silico Trial, the simulations for Dofetilide were run offline, as Dofetilide was not available in the online environment. The availability of Dofetilide is hampered by withdrawal from the market, which means it was initially not present in the online (marketed drugs) library. However, this is expected to be made possible before the end of the project.

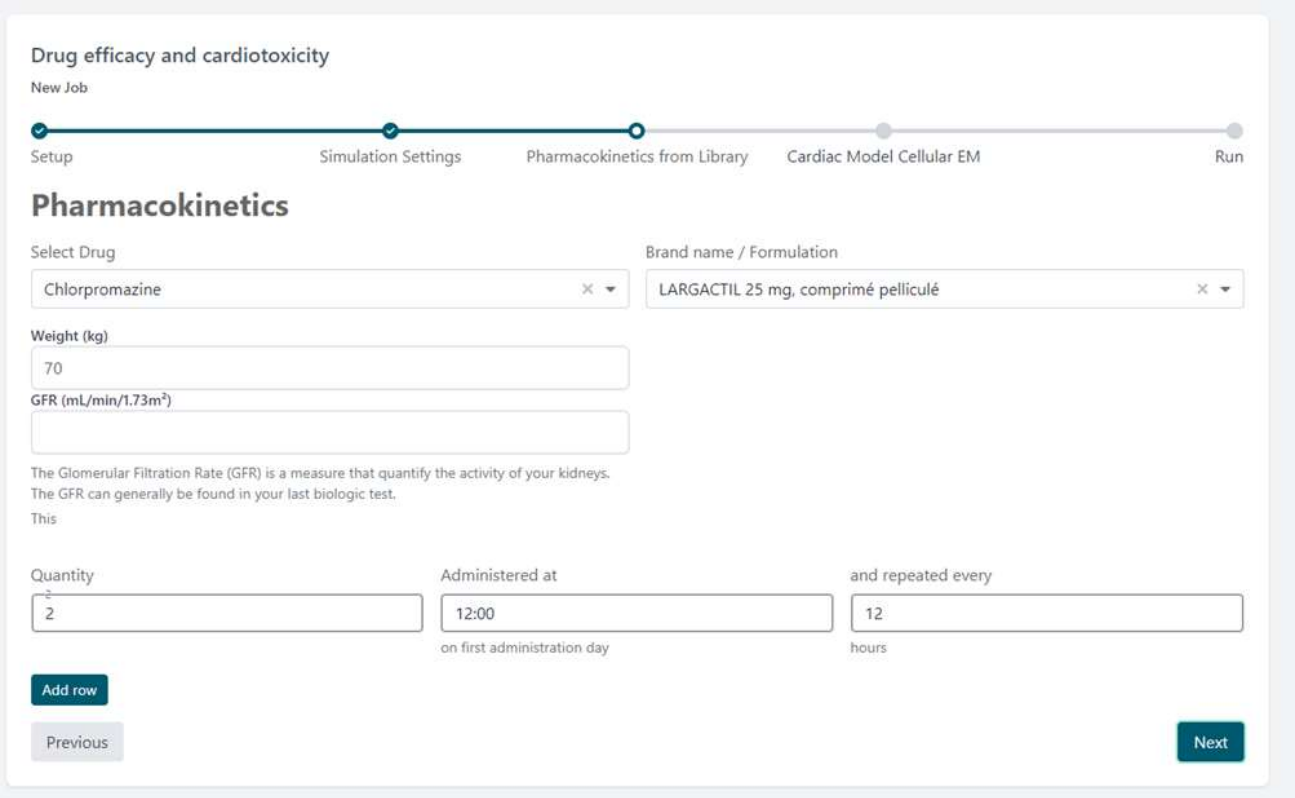


Figure 3: *Screenshot of Inputs to the pharmacokinetic model*

Step 2. Simulation

The first step, the pharmacokinetic simulation, is performed by ExactCure's server. The results are then sent directly and automatically to the SimCardioTest platform. The second step is performed in InSilicoTrials' cloud-based simulation environment. Depending on simulation complexity, the simulation can take minutes – hours.

Step 3. Output visualization

Each completed simulation can be viewed via the user dashboard. The results pages differ depending on which models the user has chosen to use, but include graphical time courses and tabular and graphical biomarkers data. The electrophysiological workflows include the final classification of the drug under this concentration as safe or unsafe.

Step 4. Evaluation

The TdP-risk classifiers described in section 3.1 were also implemented in the web interface, allowing the user to assess quickly whether the envisioned usage of the drug is safe or unsafe for the defined patient population.

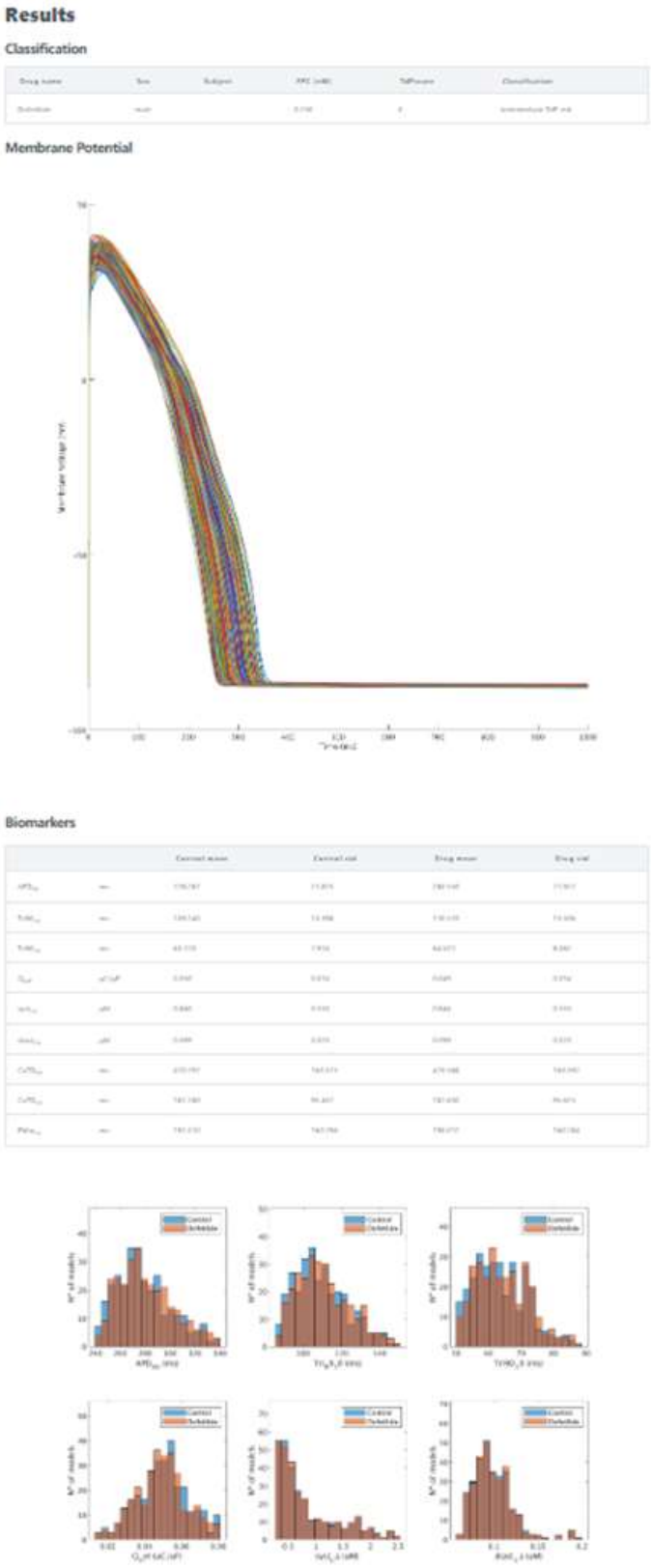


Figure 4: *Screenshot of simulation results*

5 Results

The results we present in the following document have been obtained offline during the development phase of the in-silico trial steps. In the previous section, we showed that most of these steps were implemented in the platform and simulations running online obtained identical results. However, unexpected problems delayed the implementation of the web interface and only one basic test was performed online. The results of a complete in-silico trial to assess Dofetilide are explained below.

PK output is a graph depicting the time course of the drug plasma concentration. In Figure 5, Dofetilide concentration evolves over 350 hours, enough time to achieve steady state. The same dosage regimen was metabolized differently in men and women, which explains that females presented a larger maximum plasma concentration (3.75 vs 3.30 µg/L), which was then translated to a larger EFTPC.

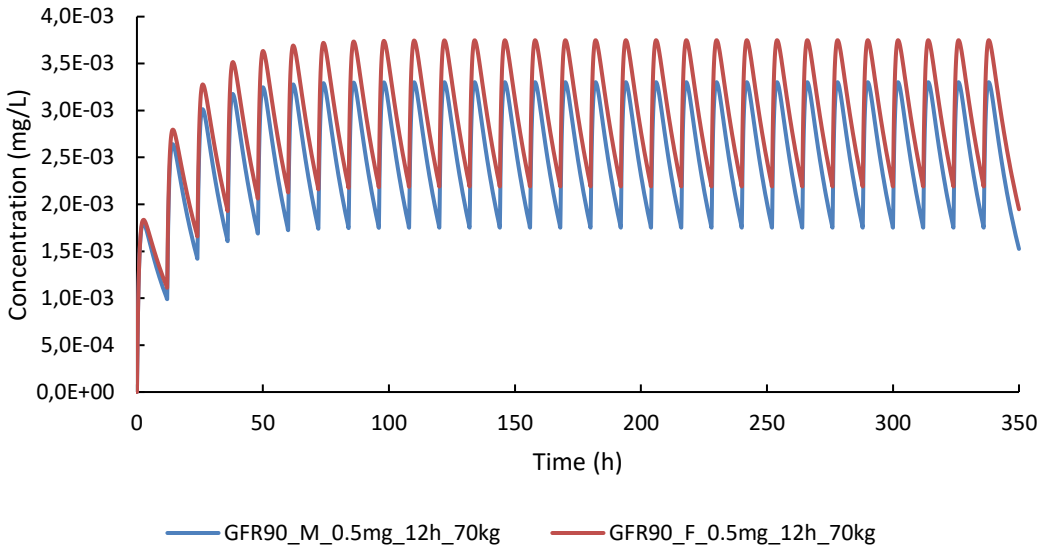


Figure 5: *Time course of Dofetilide plasma concentration***.**

These PK simulations were performed offline because non-marketed drugs in France, such as Dofetilide, were excluded from the PAPI to which the platform connect to run PK tests. However, the platform is prepared to simulate PK models, as it can use marketed drugs, and it is expected to include Dofetilide model by the end of the project so we can show a complete in-silico trial online.

The EP outputs after simulations in a generic myocyte are illustrated in Figure 6, from which 6 biomarkers were quantified. Compared to control, Dofetilide caused an increase of APD90, Tri90_50 and systCa, and reduced qNet, CaTD90 and EMw_Ca. Variations increased with concentration and biomarker values can be found in the Appendice for ease of reference.
The classifier determined that from EFTPC (Test #1) to the minimum therapeutic concentration (Test #2) conditions were proarrhythmic and therefore unsafe. The threshold between unsafe and safe was found at FPC equal to 0.25 nM for Dofetilide. A fourth test in which drug levels were reduced below the safe concentration limit (0.1 nM) confirmed the absence of TdP-risk. The results of these tests are summarized in Table 5.

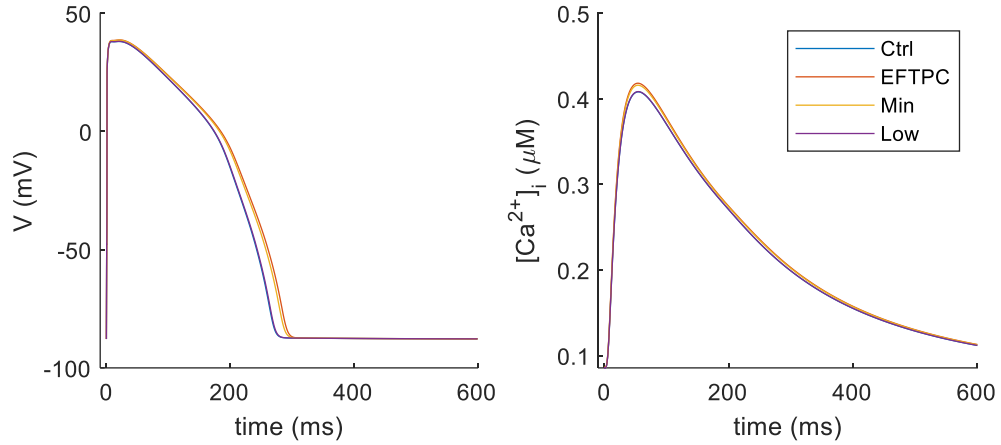


***Figure 6**: Effect of Dofetilide concentration on action potential and Ca2+ transient in a generic myocyte model.*

Table 5: TdP-risk classification of Dofetilide concentration evaluated in a generic cell.

Test	Name	FPC (nM)	Tqnet	Ttriang	Tx	Class
#1	**EFTPC**	2.62	0.446	2.019	1	Unsafe
#2	**Minimum Therapeutic**	1.59	0.572	1.698	1.585	Unsafe
#3	**Max safe C**	0.25	0.894	1.136	10	Safe
#4	**Low [C]**	0.10	0.954	1.056	25.119	Safe

Simulations in a population of cells resulted in electrophysiological variability, including action potentials of different lengths (Figure 7). The stratification in male and female subpopulations showed that women APD was longer, a recurring condition with every Dofetilide dose. Changes in 9 calculated biomarkers were reported in the Appendix, where the effect of reducing drug concentration and differences between subgroups can also be observed. However, to determine TdP-related safety conditions, machine learning techniques that processed all the data were needed. Results of the main tests performed during the iterative process to find the maximum concentration of Dofetilide to assure TdP-related safety are shown in Table 6 The ternary classifier predicted high TdP-risk for the whole population subjected to therapeutic Dofetilide doses, which means that both male and female patients would need to prioritize safety at the expense of medication benefits. The maximum safe concentration estimated with one cell simulation was identified as intermediate risk in the population, and low TdP-risk was only achieved by reducing concentration to lower levels. Despite the biomarker differences between male and female subpopulations, in terms of TdP-risk differences were very subtle. Only one scenario, out of the 4 concentrations tested in the in-silico trial, revealed risk differed between sexes. With a Dofetilide FPC of 0.1 nM, low TdP-risk conditions were estimated for males but the risk remained intermediate for females.

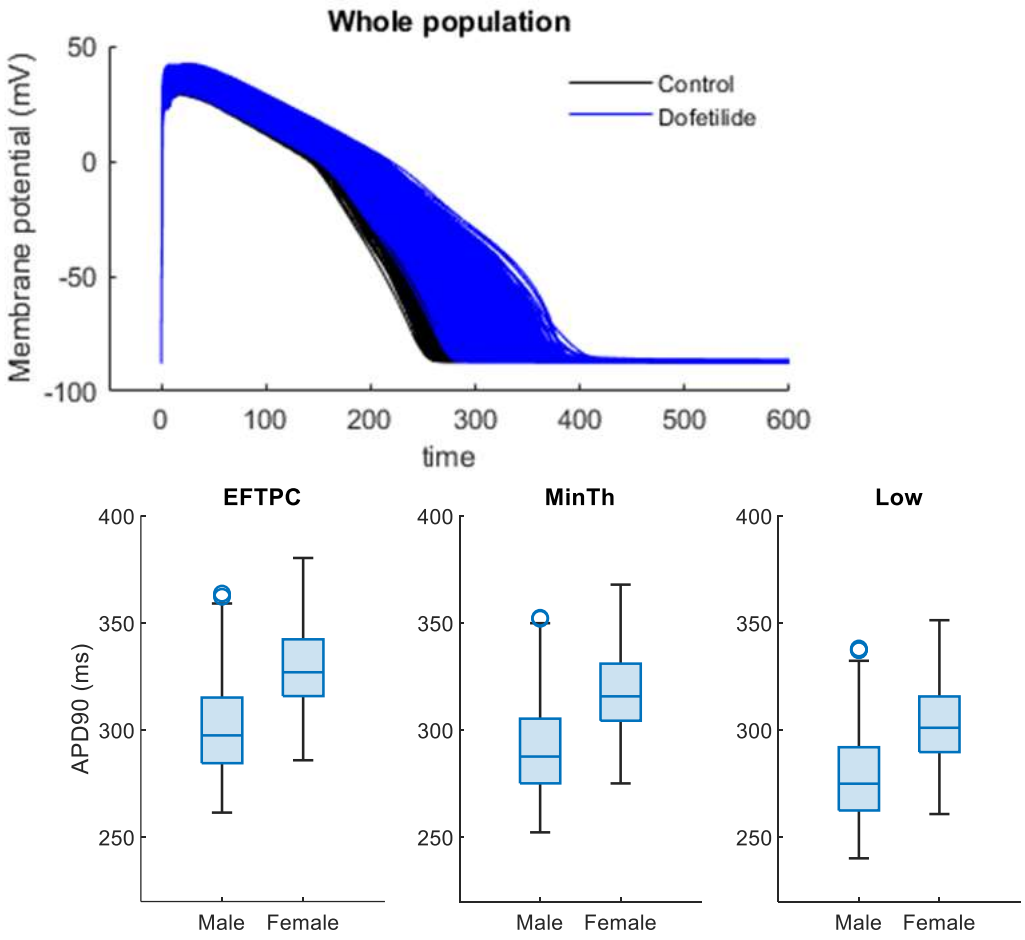


Figure 7: *Effect of Dofetilide on a population of action potentials and quantification of duration (APD90). Stratification of patients according to variable sex for different concentrations.*

Table 6: TdP-risk of Dofetilide dose evaluated in different populations.

Patient		FPC (nM)	TdP-score	Class
Test #1 EFTPC				
Male	GFR90_M_0.5mg_12h_70kg	2.62	1	High
Female	GFR90_F_0.5mg_12h_70kg	2.97	1	High
Test #2 Minimum Therapeutic [C]				
Male	-	1.59	0.517	High
Female	-	1.59	0.920	High
Test #3				
Male	-	0.25	0	Intermediate
Female	-	0.25	0	Intermediate
Test #4 Low [C]				
Male	-	0.10	-0.527	Low
Female	-	0.10	-0.473	Intermediate

Risk scale: High, Intermediate and Low risk.

Apart from the biomarkers and classification results obtained with cellular simulations, the use of a three-dimensional electromechanical model provides additional information of drug effects. Tension twitches and the stretch of cells are new signal outputs, although in the case of Dofetilide, the alteration of these parameters was not significant (
Figure *8*).The resulting simulation video of the contractile wedge allows to visualize the evolution over time and the snapshot of Figure 9 illustrates the maximal shortening in the longitudinal direction (systolic contraction) and the displacement value in each point of the cardiac tissue. The similar results between control and Dofetilide confirm that this drug does not alter contractility. The pseudo-ECG recorded from the wedge (Figure 10) demonstrate the effects of Dofetilide in increasing the QT interval.

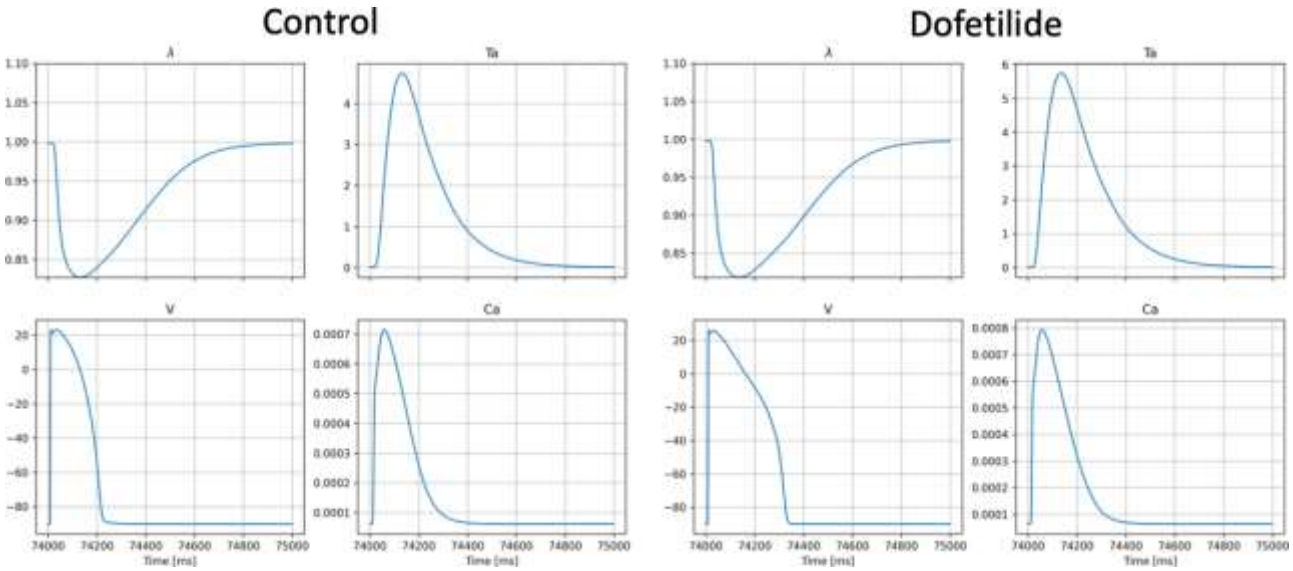


Figure 8: *Cellular mechanical effects of Dofetilide obtained in a cardiac wedge.*

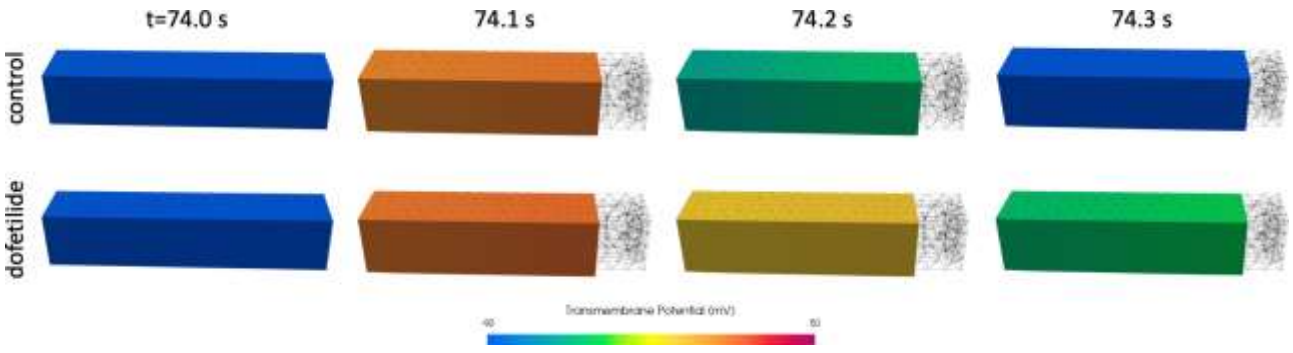


Figure 9*: Sample simulation of contraction in the cardiac wedge.*

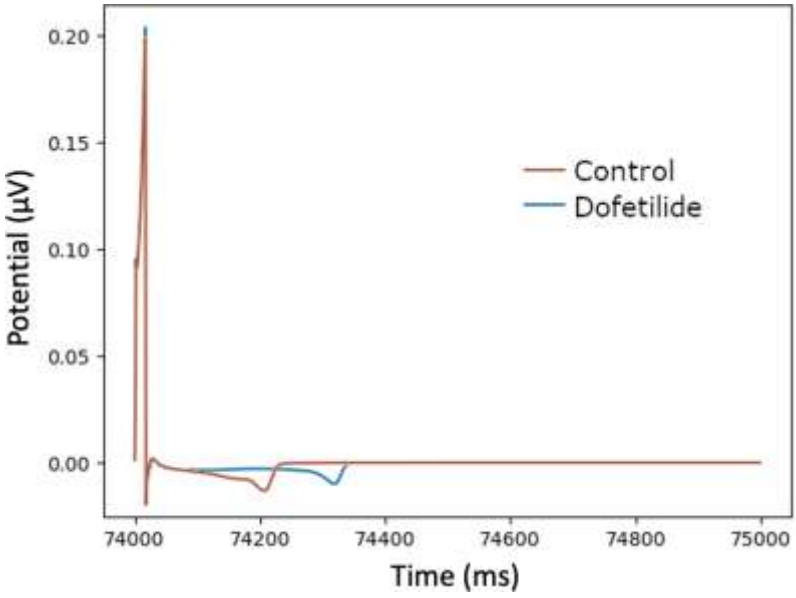


Figure 10: *Pseudo-ECG recorded from wedge*

Regarding new biomarkers, conduction velocity (CV), fractional shortening (FS) and QT interval were obtained from the electrical propagation and deformation of cardiac tissue, respectively (Table 7). In agreement with previous results, Dofetilide did not alter FS and showed the corresponding prolongation of the ECG.

Table 7: Electromechanical biomarkers from the cardiac wedge.

Patient	CV (m/s)	FS	QT(ms)
Control	0.587	0.870	202
Dofetilide	0.587	0.909	317

All these 3D results were obtained offline, but we plan to implement these tests into the platform by the end of the project to complete the in-silico trial tool.

As future work, it is planned to incorporate biomarkers from 3D simulations to the TdP-risk classifier. This study is still under development, and once finished, the benefits of including new complex data need to be evaluated.

6 Conclusions and Discussion

This report shows the steps to assess TdP-risk of molecules and determine the optimal dose to ensure safety through computer modelling and simulation. The use of patients' characteristics in the in-silico trial allowed to adjust posology to subpopulations and outperform the results of clinical trials that are limited to test in reduced and specific groups.

The example in-silico trial with Dofetilide showed that it was not possible to balance safety and efficacy for the entire population, suggesting that other pharmacological alternatives should be taken. Indeed, European agencies have withdrawn it from the market and other antiarrhythmic medicines are prescribed. In case of testing new molecules, this type of prediction would suggest to discard the compound from a safety point of view.

The differences between male and female subpopulations were very subtle. Given the elevated TdP-risk of Dofetilide at therapeutic doses for the whole population, the distinction between subgroups was not relevant. Nevertheless, reduced plasma concentrations were used to verify that the in-silico trial was able to predict different TdP-risk labels according to the characteristics of the populations, and showed that women presented higher risk than men.

With this example, we demonstrate that the in-silico trial tool developed within the SimCardioTest project fulfills the expected objectives. It can be used as a complementary tool to assess drug safety during the early phases of pharmacological development. Although additional features, such as electromechanical indices and the use of whole heart geometries might improve predictions, the current approach should not be underestimated, as it is paving the way to the use of in-silico methods.

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Appendices

Table 8: Biomarkers in a generic cell after different Dofetilide interventions.

Test	Name	FPC (nM)	APD90 (ms)	Tri90_50 (ms)	qNet (µC/ µF)	syst Ca (µM)	CaTD90 (ms)	EMw_Ca (ms)
#0	**Control**	**-**	263.4	51.7	5.00E-02	0.408	548.5	285.1
#1	**EFTPC**	2.62	288.8	58.3	4.46E-02	0.421	546.8	257.9
#2	**Minimum Therapeutic**	1.59	279.3	55.8	4.65E-02	0.416	547.4	268.2
#3	**Max safe C**	0.25	266.0	52.4	4.94E-02	0.409	548.4	282.4
#4	**Low [C]**	0.10	264.4	52.0	4.98E-02	0.408	548.5	284.0

Table 9: Biomarkers in a the population after different Dofetilide interventions (part I).

Population	FPC (nM)	APD90 (ms)	Tri90_50 (ms)	Tri90_30 (ms)	qNet (µC/ µF)
Ctrl Male	**-**	278.3 ± 21.9	109.1 ± 13.4	63.7 ± 8.0	4.98E-02 ± 1.38E-02
Ctrl Female	**-**	302.8 ± 18.6	129.6 ± 11.8	79.8 ± 8.4	3.73E-02 ± 1.43E-02
1-EFTPC					
Male	2.62	301.5 ± 22.4	123.5 ± 14.5	71.6 ± 8.9	4.52E-02 ± 1.42E-02
Female	2.97	333.3 ± 19.6	149.9 ± 13.2	91.8 ± 10.0	3.14E-02 ± 1.49E-02
2-Minimum Therapeutic [C]					
Male	1.59	292.7 ± 22.2	118.0 ± 14.1	68.6 ± 8.5	4.68E-02 ± 1.40E-02
Female	1.59	319.4 ± 19.1	140.6 ± 12.6	86.3 ± 9.2	3.38E-02 ± 1.46E-02
3-Maximum safe [C]					
Male	0.25	280.5 ± 21.9	110.6 ± 13.5	64.5 ± 8.1	4.90E-02 ± 1.38E-02
Female	0.25	305.3 ± 18.7	131.3 ± 12.0	80.9 ± 8.5	3.64E-02 ± 1.43E-02
4-Low [C]					
Male	0.10	279.1 ± 21.9	109.7 ± 13.4	64.0 ± 8.0	4.93E-02 ± 1.37E-02
Female	0.10	303.7 ± 18.6	130.2 ± 11.9	80.2 ± 8.4	3.67E-02 ± 1.42E-02

Values expressed as mean±SD

Table 10: Biomarkers in the population after different Dofetilide interventions (part II).

Population	FPC (nM)	syst Ca (µM)	diast Ca (µM)	CaTD90 (ms)	CaTD50 (ms)	EMw_Ca (ms)
Ctrl Male	-	0.840 ± 0.550	0.099 ± 0.023	470.8 ± 163.6	187.8 ± 95.5	192.5 ± 160.8
Ctrl Female	-	0.914 ± 0.572	0.102 ± 0.026	463.4 ± 151.9	180.0 ± 91.0	160.6 ± 146.6
1-EFTPC						
Male	2.62	0.863 ± 0.553	0.100 ± 0.023	469.2 ± 163.1	186.5 ± 97.2	167.7 ± 160.2
Female	2.97	0.941 ± 0.566	0.103 ± 0.026	462.2 ± 149.9	177.9 ± 92.6	128.9 ± 144.4
2-Minimum Therapeutic [C]						
Male	1.59	0.855 ± 0.552	0.100 ± 0.023	469.8 ± 163.3	187.0 ± 96.6	177.1 ± 160.5
Female	1.59	0.930 ± 0.571	0.103 ± 0.026	462.5 ± 151.0	178.8 ± 91.9	143.0 ± 145.5
3-Maximum safe [C]						
Male	0.25	0.844 ± 0.553	0.099 ± 0.023	470.6 ± 163.6	187.6 ± 95.7	190.0 ± 160.8
Female	0.25	0.919 ± 0.576	0.102 ± 0.026	463.0 ± 151.8	179.7 ± 91.1	157.7 ± 146.5
4-Low [C]						
Male	0.10	0.843 ± 0.553	0.099 ± 0.023	470.6 ± 163.6	187.7 ± 95.5	191.5 ±160.8
Female	0.10	0.918 ± 0.577	0.102 ± 0.026	463.1 ± 151.9	179.8 ±91.1	159.4 ± 146.6

Values expressed as mean±SD

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