# SILICOFCM

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# Executive summary

SILICOFCM is a multi-modular, innovative *in silico* clinical trials solution for the design and functional optimization of whole heart performance and monitoring effectiveness of pharmacological treatment, with the aim to reduce animal studies and human clinical trials. The SILICOFCM platform is based on the integrated multidisciplinary and multiscale methods for analysis of patient-specific data and development of patient-specific models for monitoring and assessment of patient condition through the course of a disease.

One of the aims of the SILICOFCM project is to provide a respectable *in silico* solution with developed computational cloud platform in all areas and at different levels of health care. The platform combines data from multiple data sets collected at multiple scales with sophisticated and continuously developing computational models, therefore, following the information flow across all length scales from a gene mutation to organ dysfunction.

To fulfil the project objectives and the roadmap goals, the presented document corresponds to revised D8.1 – "Workflow for drug testing", the first deliverable of WP8 – "Report to FDA or EMA". The deliverable is the outcome of Task 8.1 – "Development workflow assistant for EMA/FDA approval". This document presents the result of the integrated approach for drug testing by deploying the cloud-based SILICOFCM platform, its tools and modules. The performed work is related to WP4, WP5 and WP6. The document is closely related to revised D8.2 "Computational pipelines for drug testing" and should be read together. In addition, the document is interlinked with D8.3 "Interface drug database", as well as D8.4 "Development report tool".

Towards this final aim, all along the course of the project an extensive analysis of the technical and regulatory constraints for the adoption of the platform has been conducted and continuously updated, as the field of modelling and simulations is rapidly progressing, and is hereby described.

The workflows of multiple modules implemented in the SILICOFCM computational cloud platform are designed for the optimization of whole heart performance and monitoring of effectiveness of pharmacological treatment. This approach is suitable for accelerating new drug development or for testing drugs used in clinical practice. This document presents the concept and architecture of drug testing, as well as different scenarios of drug testing which are created and performed by deploying the SILICOFCM tools. In addition, it describes the drug database and future challenges.

From the analysis of the context, a proposed road map for the platform adoption is designed, with a concrete development plan based on the maturity of the available technology – framed in the current regulatory context – and following a step-wise approach: "SILICOFCM today" and "SILICOFCM tomorrow". The "SILICOFCM today" is focused on the use of the SILICOFCM platform as a research tool/decision-support system in the early phase of development of new drugs for FCM, as well as for risk stratification of FCM patients. The "SILICOFCM tomorrow" has a more ambitious goal of performing *in silico* trials as a reliable source of information that could be a relevant component of a regulatory submission.

This document is structured as follows:

**Section 1 – Introduction** presents the overview of the SILICOFCM concept, the purpose and the scope of this deliverable, and actions implemented within Task 8.1 in order to be in compliance with and address the related Description of Action.

**Section 2 – The regulatory context** analyses the general requirements for software as a medical device and presents possible domains of the SILICOFCM platform in scope of *in silico* trials for drug testing. The model types and related documentation that are treated for drug testing standards are shortly described. This section also includes the definition of Digital twins and Virtual population.

**Section 3** – **The computational cloud platform** describes the SILICOFCM architecture which consists of five layers: i) Hardware, ii) Security, iii) Workflow, iv) Back-end, and v) Front-end. The workflow management is organised following FAIR data principles. Also, the SILICOFCM running drug testing workflows were implemented on the AWS (Section 3.3).

**Section 4 – SILICOFCM Workflow for drug testing** presents deployed architecture and concept of integrated SILICOFCM tools for drug testing, covering also three different scenarios for effects of drugs on heart function using MUSICO as the core tool: i) drugs that modulate Ca2+ transients, ii) drugs that affect changes in kinetic parameters, and iii) drugs that affect changes in macroscopic parameters.

**Section 5 – Drug database** describes the use of Minerva Hypertrophic Cardiomyopathy (HCM) map in the analysis of key intracellular signalling pathways, and presents the ventricular cardiomyocyte model.

**Section 6 – SILICOFCM modules for drug testing** describes deployment of MUSICO tool and PAK solver tool in the workflow for drug testing. This section also presents drug influence on the ECG simulation and comparison with ECG clinical measurement. Finally, this section includes the approach for drug testing using Alya Solver tool.

**Section 7 – Regulatory bodies** briefly describes EMA, FDA and notified bodies, as well as communication with EMA and FDA.

**Section 8 – Roadmap to the adoption of SILICOFCM platform** analyses the classification of software as a medical device, and presents the initial development plan for adoption of the SILICOFCM platform.

**Section 9 – Deviation from the work plan** describes the deviations from the work plan.

**Section 10 – Conclusions** presents the conclusions of this document.

**Section 11 – References** presents the list of references used for making the final version of this deliverable.

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# List of Figures



# List of Abbreviations

| Abbreviation | Explanation | Abbreviation | Explanation |
|---|---|---|---|
| ACE | Angiotensin-Converting Enzyme | HPC | High Performance Computing |
| ADT | AutoDockTools | ICVDV | Institute for Cardiovascular Diseases Vojvodina |
| ANP | Atrial Natriuretic Peptide | IMDRF | International Medical Device Regulators Forum |
| APD | Action Potential Duration | IaaS | Infrastructure as a Service |
| ARB | Angiotensin II Type-1 Receptor Blocker | IQ | Installation Qualification |
| API | Application Programming Interface | ISO | International Organization for Standardization |
| ASME | American Society of Mechanical Engineers | LGA | Lamarckian Genetic Algorithm |
| ASME VV-40-2018 | Assessing Credibility of Computational Modeling through Verification and | LSTM | Long Short-Term Memory |

| | | | |
|---|---|---|---|
| | Validation: Application to Medical Devices | | |
| AT1 | Angiotensin Receptor 1 | GMP | Good Manufacturing Practice |
| AT2 | Angiotensin Receptor 2 | GRU | Gated Recurrent Unit |
| AZM | Azithromycin | GSP | Good Simulation Practices |
| AWS | Amazon Web Services | HCM | Hypertrophic Cardiomyopathy |
| BNP | Brain Natriuretic Peptide | HCQ | Hydroxychloroquine |
| CDS | Clinical Decision Support | HF | Heart Failure |
| CM&S | Computational Modelling and Simulations | LV | Left Ventricle |
| CNN | Convolutional Neural Network | MDD | Medical Device Data |
| CNP | C-Type Natriuretic Peptide | MDR | Medical Device Regulation |
| COMBINE | Computational Modelling in Biology Network | MDSW | Medical Device Software |
| COU | Context of Use | MP | Mijailovich - Prodanovic |
| CFD | Computational Fluid Dynamics | MR | Mitral Regurgitation |
| CWL | Common Workflow Language | MUSICO | MUscle SImulation COde |
| DBP | Diastolic Blood Pressure | MyBP-C | Myosin Binding Protein C |
| DCM | Dilated Cardiomyopathy | NB | Notified Bodies |
| DoA | Description of Action | ODE | Ordinary Differential equations |
| DNS | Domain Name Service | OQ | Operational Qualification |
| DQ | Design Qualification | PDB | Protein Data Bank |
| ECG | Electrocardiogram | POCASA | POcket-CAvity Search Application |
| ECS | Elastic Container Service | PQ | Performance Qualification |
| EDV | End-Diastolic Volume | PV | Pressure - Volume |
| EESC | European Economic and Social Committee | RAAS | Renin-Angiotensin Aldosterone System |
| EF | Ejection Fraction | RU | Regulatory Unit |
| EMA | European Medicines Agency | SaMD | Software as a Medical Device |
| EKS | Elastic Container Service for Kubernetes | TnC | Troponin C |
| ESV | End-Systolic Volume | UHREG | University Hospital Regensburg |
| FCM | Familial Cardiomyopathy | UNEW | Newcastle University |
| FDA | Food and Drug Administration | UNIFI | University of Florence |
| FE | Finite Element | VPC | Virtual Private Cloud |
| FES | Functional Engine Server | VPM | Virtual Population Model |
| FMBG | Faculty of Medicine, University of Belgrade | WDL | Workflow Description Language |
| GCP | Good Clinical Practice | WT | Wild Type |
| GLP | Good Laboratory Practice | | |

# 1 Introduction

The SILICOFCM project aims to develop a computational platform for *in silico* clinical trials of Familial cardiomyopathies (FCMs). The platform takes into consideration a comprehensive list of patient-specific features (genetic, biological, pharmacologic, clinical, imaging and patient specific cellular aspects) capable of optimizing and testing medical treatment to maximize positive therapeutic outcome, avoiding adverse effects and drug interactions, preventing sudden cardiac death, shortening time between the drug treatment commencement and the desired result.

The main result of the SILICOFCM project will be a multi-modular, innovative *in silico* clinical trials solution for the design and functional optimization of whole heart performance and monitoring effectiveness of pharmacological treatment, with the aim to reduce animal studies and human clinical trials. The SILICOFCM platform is based on the integrated multidisciplinary and multiscale methods for the analysis of patient-specific data and development of patient-specific models for monitoring and assessment of patient condition through the progression of disease.

One of the aims of the SILICOFCM project is to become a respectable *in silico* solution with developed computational cloud platform in all areas and levels of health care, by combining the data from multiple data sets collected at multiple scales with sophisticated and continuously developing computational models and, therefore, following the information flow across all length scales from a gene mutation to organ dysfunction. Thus, a deep analysis has been conducted to frame the path towards this final objective of the project.

The analysis, described in this deliverable, begins with an overview of the context, starting from the current regulatory steps for the development of medical devices, in general, and more specifically the ones to be followed for the development and marketing approval of drugs for cardiomyopathies. Then, an analysis of the current status for the adoption of *in silico* methods is crafted, from the different modelling solutions to the requirements for the credibility and acceptability of these methods from scientific and regulatory point of views.

From the analysis of the context, a proposed road map for the platform adoption is designed, with a concrete development plan based on the maturity of the available technology – framed in the current regulatory context – and following a step-wise approach: “SILICOFCM today” and “SILICOFCM tomorrow”. The “SILICOFCM today” is focused on the use of the SILICOFCM platform as a research tool/decision-support system in the early phase of development of new drugs for FCM, as well as for risk stratification of FCM patients. The “SILICOFCM tomorrow” has a more ambitious goal of performing *in silico* trials as a reliable source of information that could be a relevant component of a regulatory submission.

The presented deliverable should be read together with the deliverable D8.2 “Computational pipelines for drug testing”.

## 1.1 Relation to the DoA

The following table presents the DoA description of Task 8.1 and how this deliverable addresses the description of the Task.

| DoA Task Description | Addressed by D8.1 |
|---|---|
| This task will define workflow for computational modelling for basic drug testing for FCM. The purpose of these tests is to evaluate aspects of the long-term patient integrity for FCM under different conditions. BIOIRC together with other partners UOI, IIT, BSC, R-Tech, SBG, UW, UL, ICVDV will define workflow for EMA/FDA approval. The idea is to reduce clinical trials and to reduce animal experiments with new drugs for FCM. | The presented document addresses the DoA description in the following:<br>The computational cloud platform is described in Section 3. The workflows for computational modelling for basic drug testing for FCM are described in Section 4. This section includes the concept and architecture of, as well as the scenarios for drug testing and effects on heart functions. Two different drugs have been included in D8.1: i) Disopyramide, and ii) Entresto®. In addition, the drug database is described in detail (Section 5).<br><br>In collaboration of technical partners, SILICOFCM modules for drug testing including MUSICO, PAK and Alya solver tools are presented (Section 6). Three different use scenarios of PAK solver tool are given, while more details and use scenarios for MUSICO tool are included in D8.2. The Section 6 also includes the main aspect of drug influence on the ECG simulation (Section 6.4).<br><br>Finally, the regulatory context (Section 2) and notified bodies (Section 7) are analysed in detail. This resulted in the creation of the initial roadmap to the adoption of the SILICOFCM platform (Section 8). |

## 1.2 Reviewer's recommendations and how we addressed them

After the project review meeting that was held on July 5th 2021, the consortium received the report which stated that the deliverable was rejected and sent back for updates. The reviewers' comments and suggestions are shown in the following table, as well as the consortium's responses.

| Reviewer's recommendations | How we addressed them? |
|---|---|
| Some of the results presented in D8.1 and D8.2 (left ventricular P-V loops) are seriously questionable and do not represent real clinical data. A significant review should be considered highlighting the assumptions made, current limitations and alternative approaches. | After fixing some bugs in the software, simulations for the left ventricular P-V loops are running again and diagrams that are more realistic are included in D8.2. All the cases/examples are in D8.2 and all workflows are in D8.1 as reviewers suggested. |
| The ECG dataset used in one of the presented studies is of acceptable size (102 HCM patients, 153 ECG recordings). However, the population of patients engaged in the rest of the studies is not clear, which raises concerns regarding the reliability and generalization of the results. This needs to be clearly specified. | The UNEW is a coordinator of the clinical prospective study contained in WP3 and therefore responsible for data collection, analysis, interpretation and presentation. At the time of the progression review meeting, participating clinical centres emailed data to the UNEW, which collated data from all the centres, screened them for outliers and entered them into a single database. The numbers indicated in the review comment i.e. 102 and 153 relate to the patients assigned to an intervention (n=102) and a total number of patients consented into the study (n=153) at the time for the progression review meeting. Data for all patients consented into the study will be further screened and included into final analysis once the data collection phase for clinical study is completed.<br><br>It is added in Section 6.4. |
| If there are any existing standards on the drug testing processes, these should be mentioned in Chapter 2. | The model types and related documentation that are treated for drug testing standards are desribed in Section 2.2. |
| The deliverable needs to also present the actions that have taken place to communicate with the EMA and FDA regulatory bodies in order to promote the SILICOFCM workflow for drug testing. This should be inserted as an additional section under Chapter 6. | The latest actions that have been performed by SILICOFCM consortium towards communication with EMA and FDA regulatory bodies, acceptance of SILICOFCM results and CM&S are outlined in Section 7.2.<br><br>So far, through the In Silico World group we have had a meeting with various FDA officers, and a formal meeting with the Innovation Task Force of EMA. Note that sections numbering differs from the v1.0 of the deliverable. |

| | |
|---|---|
| It is assumed that all figures in D8.1 are generated within SILICOFCM. If this is not the case, the source of these figures is missing and should be clearly added. | All figures are generated with the SILICOFCM platform. |

# 2 The regulatory context

According to the exploitation plan, the aim of the SILICOFCM project is to develop an *in silico* clinical trial platform for the design and functional optimization of whole heart performance and monitoring effectiveness of pharmacological treatment, with the aim to reduce animal studies and human clinical trials. The term *in silico* trials refers to the use of computer simulation to assess the safety and/or efficacy of new healthcare products, whether medical devices, medicinal products, or others.

The regulatory process aims to provide marketing authorisation only to those medical products for which the applicant can demonstrate justified claims of safety and efficacy and/or performance. Depending on the type of medical product and its risk class, such claims must be corroborated with evidence of safety and efficacy obtained with a set of controlled experiments conducted *in vitro* or *ex vivo*, *in vivo* on animals, or *in vivo* on humans, with multiple clinical trials involving progressively growing numbers of participants.

In Silico Trials aim to reduce, refine, or replace these experiments[1]:

**-Reduce** means to reduce the number of *in vitro* experiments or those involving living subjects (animals or humans), their duration, or the number of experimental subjects (animals or humans) involved in the experiment, or the number of measurements performed during the experiment.

**-Refine** means to revise the study design in order to eliminate or relieve the suffering of the animals involved, or the risks for the humans involved in the experiments; or to shift the experiment to non-animal species, in accordance with animal experimentation ethics. For in vitro experiments and animal experiments, refine also means improving the ability of the experiment to predict the results of the human experimentation[2].

Current human cardiac electrophysiology models integrate detailed information on the dynamic processes underlying cardiac electrical excitation from subcellular to whole organ levels[3]. Modelling and simulation studies have played a central role in the discovery of cardiac arrhythmia mechanisms[4].

## 2.1 General requirements for software as a medical device

The model credibility evaluation process has been introduced by the ASME VV-40-2018 standard.

The ASME VV-40-2018 standard, 'Assessing Credibility of Computational Modeling through Verification and Validation: Application to Medical Devices', introduced the risk-informed credibility assessment framework shown in Figure 1. ASME V&V standards provide the guidance that helps practitioners better assess and enhance the credibility of their computational models.

The credibility assessment process begins with a question of interest, which is generally framed around a specific aspect of the functional performance of a medical device that is linked to its safety and/or efficacy.

Data generated through a variety of preclinical or clinical experiments are actually answers to the question of interest. These questions can be answered partly or in total with modelling and simulation.

[1] Viceconti, M., Emili, L., Afshari, P., Courcelles, E., Curreli, C., Famaey, N., ... & Pappalardo, F. (2021). Possible Contexts of Use for In Silico trials methodologies: a consensus-based review. arXiv preprint arXiv:2103.15065.
[2] https://insilico.world/community
[3] Niederer, S. A., Lumens, J., & Trayanova, N. A. (2019). Computational models in cardiology. Nature Reviews Cardiology, 16(2), 100-111.
[4] Noble, D. (2011). Successes and failures in modeling heart cell electrophysiology. Heart Rhythm, 8(11), 1798-1803.

We can say that the 'Context of Use' (COU) is the term used by the standard to specify the role of modelling and simulation in addressing the question of interest.

Verification is performed to determine if the computational model fits the mathematical description. Validation is implemented to determine if the model accurately represents the real world application. Uncertainty quantification is conducted to determine how variations in the numerical and physical parameters affect simulation outcomes.

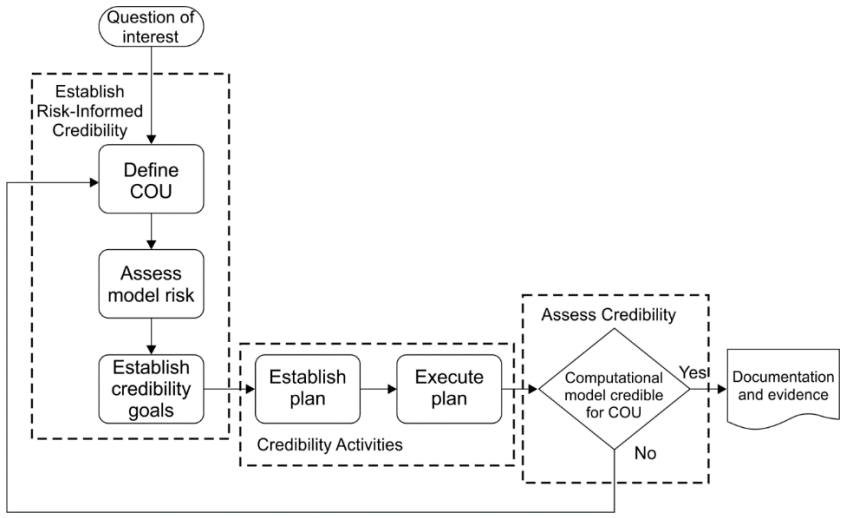


**Figure 1.** The risk-informed credibility assessment framework of ASME V&V40-2018 (adapted from ASME V&V 40-2018).

**Software validation methods for** FDA and EMA's guidance have to fulfill the following requirements:

- **Design Qualification (DQ):** This is typically supplied by the software vendor; it documents design specifications, software requirements, functional specifications, operational specifications and vendor attributes.
- **Installation Qualification (IQ):** At this stage, your tests and documentation will confirm that the software has been installed correctly — according to your company's specifications and user requirements, the vendor's recommendations and the FDA's guidance. This goes for all hardware, software, equipment and systems.
- **Operational Qualification (OQ):** These tests establish confidence that the software will consistently perform the way it's supposed to when operating within expected ranges. These tests and results can be supplied by the vendor, since they involve standard features and security capabilities.
- **Performance Qualification (PQ):** This stage confirms that the software, as it was installed, will perform the way your company needs it to. Based on the processes and specifications outlined in the previous stages, your tests and documentation validate that the product being produced will meet FDA requirements for functionality and safety.[5]

[5] https://www.datacor.com/the-datacor-blog/fda-software-validation

## 2.2 In silico trials for drug testing with SILICOFCM

The term "In Silico Trial" indicates the use of computer modelling and simulation to evaluate the safety and efficacy of a medical product, whether a drug, a medical device, or an advanced therapy medicinal product. The aim of the SILICOFCM *in silico* trial is the design and functional optimization of whole heart performance and monitoring effectiveness of pharmacological treatment, with the aim to reduce animal studies and human clinical trials.

The SILICOFCM platform will reduce research and development costs and lead to safer medicines in the drug development process (Figure 2). It can be used for prediction of drug safety and efficacy directly in humans and, therefore, contributing to a net reduction and eventual replacement of animal research, and by providing mechanistic insights of drug action and demographic heterogeneity in drug response. The SILICOFCM platform contains multiple modules that interconnect the experiments from molecular interactions to whole heart physiological function which can be used for drug testing. Different pathways through the modules and supporting databases specific for each drug action have been described in Section 3. The user can select the drug from the drug database which further leads from molecular levels interaction and their regulation to the effects on function at the organ level. Different pathways of drug are defined, at the level of contractile proteins, at the level of regulation of transient intracellular calcium concentration and at the level tissue remodelling and/or by modulation of blood vessel elasticity, i.e. resistance to blood flow and cardiac output.

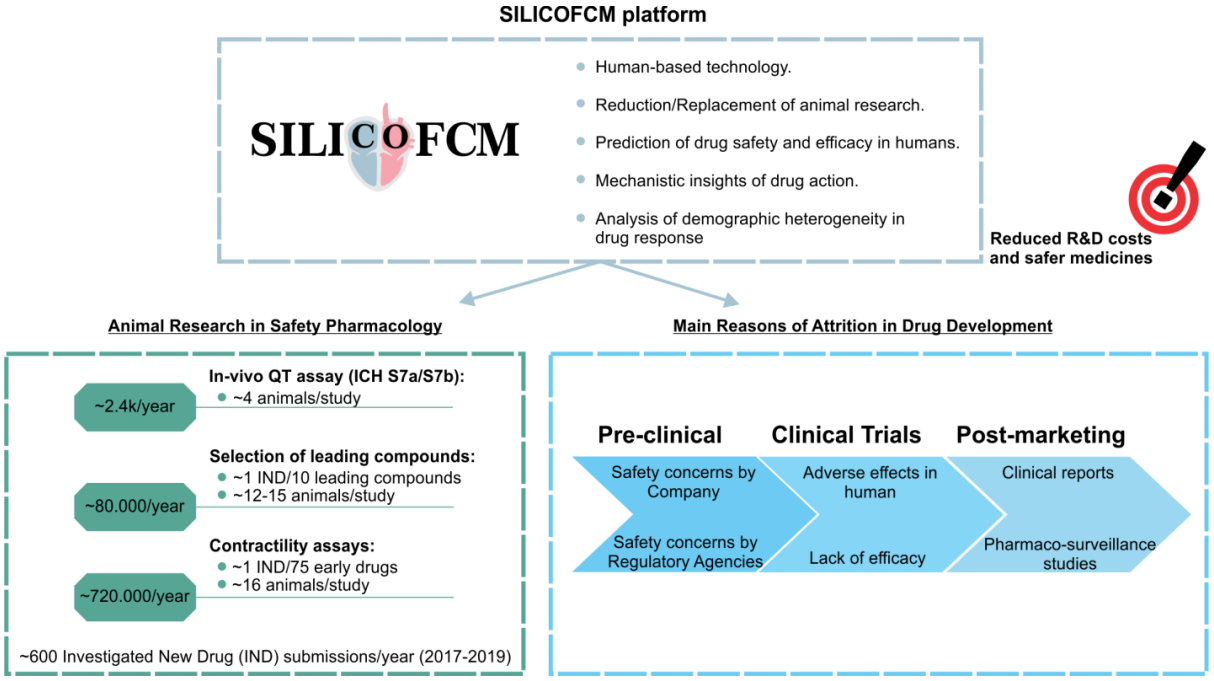


**Figure 2.** Possible domains of the SILICOFCM platform. Estimates of animal use in industry based on Investigated New Drug (IND) submissions to the FDA in the last three fiscal years (2017: 452; 2018: 675; 2019: 618; average: 582).[6]

The model types and related documentation that are treated for drug testing standards in the following text are related to drugs (Quantitative structure–activity relationship models (QSAR),

[6] Passini, E., Zhou, X., Trovato, C., Britton, O. J., Bueno-Orovio, A., & Rodriguez, B. (2020). The virtual assay software for human in silico drug trials to augment drug cardiac testing. Journal of Computational Science, 101202.

population pharmacokinetic (Pop-PK) models, Pharmacodynamic (PD) models, extrapolation models, and physiologically-based pharmacokinetic (PBPK) models )[7,8]

### 2.2.1 Drugs - QSAR

*ENV/JM/MONO(2004)24. Report from the Expert Group on (Quantitative) Structure-Activity Relationships on the Principles for the Validation of (Q)SARs. Paris, France: Organization for Economic Co-operation and Development (OECD) Expert Group on QSARs.*[9]

Quantitative structure–activity relationship models (QSAR models) are regression or classification models used in the chemical and biological sciences and engineering. Like other regression models, QSAR regression models relate a set of "predictor" variables (X) to the potency of the response variable (Y), while classification QSAR models relate the predictor variables to a categorical value of the response variable. In QSAR modelling, the predictors consist of physicochemical properties or theoretical molecular descriptors of chemicals; the QSAR response-variable could be a biological activity of the chemicals. QSAR models first summarize a supposed relationship between chemical structures and biological activity in a dataset of chemicals. Second, QSAR models predict the activities of new chemicals. QSAR models can be as simple as a statistical regression, involve molecular dynamics calculations (e.g. 3D-QSAR based on binding affinity), or very advanced machine learning models. They almost never include a mechanistic model of the physiology: they capture either the mechanistic chemistry of the drug action at the molecular scale, or build phenomenological relations with clinical endpoints.

### 2.2.2 Drugs - Pop-PK

*EMA/CHMP/EWP/185990/06. Committee for Medicinal Products for Human Use (CHMP). Guideline on reporting the results of population pharmacokinetic analysis.*[10]

Population pharmacokinetics is the study of variability in drug concentrations between individuals (healthy volunteers or patients). It comprises the assessment of variability within the population and to account for the variability in terms of patient characteristics such as age, renal function or disease state. The non-linear mixed effects modelling approach has become increasingly used for population pharmacokinetics. The EMA “Guideline on reporting the results of population pharmacokinetic analyses” implies the use of such approach. In contrast to the FDA guidance on population PK analyses, this guideline does not provide guidance on how to conduct a population PK analysis, but rather provides guidance on points to consider when presenting the results from such an analysis.

[7] EMA/219860/2020 1 June 2020: Questions and answers: qualification of digital technology-based methodologies to support approval of medicinal products.

[8] https://www.ema.europa.eu/en/documents/other/questions-answers-qualification-digital-technology-based-methodologies-support-approval-medicinal_en.pdf

[9]https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2004)24&doclanguage=en

[10] https://www.ema.europa.eu/en/reporting-results-population-pharmacokinetic-analyses

### 2.2.3 Drugs - Pharmacodynamic or exposure-response models

*FDA (CDER, CBER) 2003. Guidance for Industry: Exposure-Response Relationships — Study Design, Data Analysis, and Regulatory Applications.*[11]

The concept of exposure and response are not unequivocally defined. The broad term exposure is used to refer to dose (drug input to the body) as well as to various measures of acute or integrated drug concentrations in plasma and other biological fluids. Similarly, response refers to a direct measure of the pharmacologic effect of the drug. Response includes a broad range of endpoints or biomarkers ranging from the clinically remote biomarkers (e.g. receptor occupancy) to a presumed mechanistic effect (e.g. ACE inhibition), to a potential or accepted surrogate (e.g. effects on blood pressure, lipids, or cardiac output), and to the full range of short-term or long- term clinical effects related to either efficacy or safety.

### 2.2.4 Drugs - Extrapolation models

*EMA/189724/2018. Reflection paper on the use of extrapolation in the development of medicines for paediatrics*[12]

Extrapolation is defined as 'extending information and conclusions available from studies in one or more subgroups of the patient population (source population(s)), or in related conditions or with related medicinal products, in order to make inferences for another subgroup of the population (target population), or condition or product, thus reducing the amount of, or general need for, additional evidence generation (types of studies, design modifications, number of patients required) needed to reach conclusions'. While the focus is on extrapolation for the development of medicines in children, the underlying principles may be extended to other areas.

### 2.2.5 Drugs - PBPK

*EMA/CHMP/458101/2016. Guideline on the reporting of physiologically based pharmacokinetic (PBPK) modelling and simulation.*[13]

When reporting PBPK modelling and simulation studies, one should always provide:

- Objective and regulatory purpose
- Background information
- Qualification
- Model parameters
    - Assumptions
    - System-dependent parameters
    - Drug parameters and the drug model
- Model development
- Simulation of the intended scenario
    - Platform and drug model evaluation
    - Sensitivity analyses

[11] https://www.fda.gov/about-fda/center-biologics-evaluation-and-research-cber/transfer-therapeutic-products-center-drug-evaluation-and-research-cder

[12] https://www.ema.europa.eu/en/extrapolation-efficacy-safety-paediatric-medicine-development

[13] https://www.ema.europa.eu/en/reporting-physiologically-based-pharmacokinetic-pbpk-modelling-simulation

- Evaluation of the predictive performance of the drug model
- Results
- Discussion of the regulatory application.

In particular, with respect to model evaluation, the EMA wrote: "A comprehensive summary of the system and drug model evaluation should be provided. A thorough evaluation of the drug model is important if the model is to be used to simulate novel situations, e.g. a drug interaction or pharmacokinetics in an alternate population. An evaluation of the model should be presented in sufficient detail in the report to support confidence for regulators in the application of the model in their decision making".

*FDA/CDER draft guidance October 2020. The Use of Physiologically Based Pharmacokinetic Analyses — Biopharmaceutics Applications for Oral Drug Product Development, Manufacturing Changes, and Controls - Guidance for Industry*[14]

This guideline covers the concept of quality by design (QbD) principles and propose that the application of PBPK modelling could be expanded to pharmaceutical drug product development, manufacturing changes, and controls. It is applicable to oral formulations, only.

In addition to the general considerations (which follow a similar structure as in the previously described guideline), specific applications of PBPK modelling to support product quality are described:

1. Development of Clinically Relevant Dissolution Specifications (Method and Acceptance Criteria):
    a. Aid in Biopredictive Dissolution Method development
    b. Support Clinically Relevant Dissolution Acceptance Criteria
2. Establishment of Clinically Relevant Drug Product Quality Specifications (Other Than Dissolution)
3. Quality Risk Assessment for Pre- and Post-approval Changes and Risk-Based Biowaivers.

[14] https://www.fda.gov/vaccines-blood-biologics/biologics-guidances/recently-issued-guidance-documents

## 2.3 Computational modelling and simulations

Computational modelling is the process of representing a real-world system by means of a computer and then running the simulation by implementing a numerical scheme[15]. Computational modelling has the ability to support product development as either a stand-alone form of evidence or in conjunction with already accepted forms of evidence (bench testing, animal testing, and clinical trials).

Computational modelling and simulations (CM&S) are already used by pharmaceutical and medical device companies in the development process of new biomedical products to aid device development and design optimization, assess post-market changes or failures. Once identified, CM&S tools can then be used for redesign to address the failure mode and re-establish the performance profile with the appropriate models[16]. CM&S of the human body are also a powerful tool in biomedical research and disease modelling.

*In silico* methods are rapidly evolving as computational evidence in the regulatory decision-making process for medical devices as well as in the pharmaceutical area. However, as already commented, the utilization of modelling with regulatory agencies in Europe encounters barriers is mainly related to the lack of specific guidelines for model validation and model reporting.

The FDA is more advanced in this field and has addressed the point of model reporting releasing in 2016 a guidance that tackles the required information to judge whether a model has sufficient credibility to serve as valid scientific evidence for regulatory decision-making[17]. The guidance provides well-established structure to summarize a computational model's framework and results for review.

Another reason for the limited utilization of simulation results in regulatory submissions is the need for consensus on the evidentiary bar required to establish sufficient credibility of a computational model. The American Society of Mechanical Engineers (ASME) Verification and Validation (V&V) subcommittee on computational models of medical devices (ASME V&V 40 subcommittee), which was formed in 2011 to address this critical need, has recently released a specific guideline[18]. These two documents currently represent the pillars of regulatory reference in the US.

The regulatory impact of the model is one essential element to determine the qualification requirements, the regulatory impact being directly linked to the risk to the patient in the case the modelling predictions lead to erroneous regulatory decisions.

### 2.3.1 Digital Twins

Digital Twins will shift the current treatment selection based on the state of the patient of today to an optimized state of the patient of tomorrow[19]. This way of future medicine will be predictive, preventive, personalized, and participative.

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[15] Morrison, T. M., Pathmanathan, P., Adwan, M., & Margerrison, E. (2018). Advancing regulatory science with computational modeling for medical devices at the FDA's office of science and engineering laboratories. Frontiers in medicine, 5, 241.

[16] Morrison, T. M., Dreher, M. L., Nagaraja, S., Angelone, L. M., & Kainz, W. (2017). The role of computational modeling and simulation in the total product life cycle of peripheral vascular devices. Journal of medical devices, 11(2).

[17] Reporting of Computational Modeling Studies in Medical Device Submissions - Guidance for Industry and Food and Drug Administration Staff September 21, 2016

[18] ASME V&V 40 VERIFICATION AND VALIDATION IN COMPUTATIONAL MODELING OF MEDICAL DEVICES - Assessing Credibility of Computational Modeling through Verification and Validation: Application to Medical Devices 2018

[19] Corral-Acero, J., Margara, F., Marciniak, M., Rodero, C., Loncaric, F., Feng, Y., ... & Lamata, P. (2020). The 'Digital Twin'to enable the vision of precision cardiology. European heart journal, 41(48), 4556-4564.

Physically based modelling encapsulates all simulation methods based on our knowledge of physiology and the fundamental laws of physics and chemistry, such as solid mechanics, fluid dynamics, mass transfer and electromagnetism. These models follow a deductive approach.

Dassault Systèmes in 2020 announced a five-year extension of its ongoing *in silico* clinical trial experiment with the US Food & Drug Administration to explore how virtual or digital twins might be used in drug and device development.[20]

Currently, drug companies have only one way to find out if a new medicine works: patient-based clinical trials. However, the possibility for making virtual patients is promising to modernize the drug testing process by predicting drugs' effects.

In silico trials should mimic human physiology that pharmaceutical companies can use as a proxy for patients. In the SILICOFCM platform, researchers and clinicians can input drug data from the drug database (Section 5) into the simulated model to predict a drug's exposure in patients (scenarios given in Section 6). In this way, the safety and efficacy of the drug can be tested without any consequences for humans and animals.

*In silico* trials may also potentially protect public health. Clinical trials are the most expensive part of drug development. The SILICOFCM platform can be used to predict the safe and effective dosage before starting trials.

Another challenge facing drug makers is to state with confidence how their drug might interact with any other drugs a patient takes. The SILICOFCM platform has incorporated workflows for testing of different drugs by deploying the SILICOFCM modules and tools. Different scenarios for drug testing are given in Sections 4 and 6.

Physically based models (mechanistic) include knowledge of physiology and the fundamental laws of physics and chemistry. In the SILICOFCM platform, we integrate and augment experimental and clinical data, enabling the identification of mechanisms such as change of LF wall elasticity, blood flow, Ca2+ function and/or the prediction of outcomes, even under unseen scenarios without the need for retraining.

For a good understanding of the problem mechanistic, modelling can be an appropriate solution. On the other hand, a statistical model can find predictive relations even when the function of the problem is not understood or too complex to make a model with mechanistic approach. Statistical models encapsulate the knowledge and relations induced from the data and allow the extraction and optimal combination of different patient input, biomarkers, geometry with mathematical rules. Some good examples of statistical models applied to computational cardiology are random forests for assessment of heart failure severity[21] or Gaussian processes to capture heart rate variability[22].

Many clinical problems can be solved with a single modelling approach. However, both mechanistic and statistical models have some limitations. The integration of both of them can address these limitations. Mechanistic models are constrained by their premises (assumptions and principles), while statistical models are constrained by the observations available (the amount and diversity of data).

[20] https://www.clinicalresearchnewsonline.com/news/2020/07/13/the-role-of-virtual-twins-in-clinical-trials

[21] Guidi, G., Pettenati, M. C., Miniati, R., & Iadanza, E. (2013, July). Random forest for automatic assessment of heart failure severity in a telemonitoring scenario. In 2013 35th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC) (pp. 3230-3233). IEEE..

[22] Stegle, O., Fallert, S. V., MacKay, D. J., & Brage, S. (2008). Gaussian process robust regression for noisy heart rate data. IEEE Transactions on Biomedical Engineering, 55(9), 2143-2151.

The digital twin is in the pioneer phase. The holistic integration of a Digital Twin can go through two complementary and synergetic pathways: the first is the refinement of key decision points in the management of cardiac disease, driven by personalized mechanistic models that are informed by key pieces of patient's data; and the second is the disease-centred optimization of the patient's lifetime journey through the healthcare system, driven by statistical models being informed by the electronic health record of a large population[19]. The SILICOFCM platform integrates both mechanistic, used for 3D simulation of LV, and whole heart model as well as the ML approach for risk-stratification and prediction of HCM patients.

## 2.3.2 Virtual population

The concept of virtual patient, according to the FDA, is that of taking a physical engineering model and applying probabilistic methods to account for patient activity, patient variability in size, etc., and performing thousands of simulations to predict a given clinical endpoint. The data from the virtual patient model is then used as "prior knowledge" for designing an adaptive clinical trial where the clinical endpoint (for example, a lead fracture) is evaluated with a combination of the data from the real patients and virtual patients. This concept is being called "in silico-augmented" clinical trial. A virtual patient is an approach that allows previously collected evidence (such as digital evidence or other historical clinical evidence typically referred to as "external evidence") to inform the collection of new evidence from a clinical trial using Bayesian methodologies[15].

A different concept of virtual population is the one of creating virtual patients by mimicking the physiology of the target patients with all the variations that actual patients show. The latter is the approach that has been followed in the SILICOFCM project for the creation of the virtual population. The design, development and implementation of the SILICOFCM virtual patients repository was a continuous, iterative and collaborative process in which the clinical knowledge was translated in a unified multi repository virtual population model (VPM). The SILICOFCM VPM is an innovative approach to creating a pool of diverse virtual plausible patient representatives of the real cardiomyopathy patient population. Virtual generated clinical data, virtual experimentation generated data, virtual generated ideal LV/bi-ventricle heart geometries and 3D reconstructed patient specific heart geometries are integrated in the VPM. This approach is extensively covered in the deliverable D6.1 "Virtual FCM patients' models repository".

# 3 Computational cloud platform

The integration of the computational tools from WP4, WP5 and WP6 is set for simulation of the effect of various drugs on impearled cardiac function causing various cardiomyopathies. This approach is suitable for accelerating new drug development or for testing drugs used in clinical practice. Multiple computation modules are implemented for that purpose and integrated into the SILICOFCM platform.

## 3.1 Platform Architecture

A variety of experimental data and a system of interconnected computational tools integrated into the cloud platform enables quantitative assessment of the effects of drugs at different length and time scales. The SILICOFCM architecture is shown in Figure 3 and consists of the following layers:

1. **Hardware** - computational, storage, and networking resources provided either on-premises or as IaaS (Infrastructure as a Service). In the case of IaaS, special attention is given to VPC (Virtual Private Cloud). The appropriate firewall rules are set on both on-premises and IaaS types of deployment.
2. **Security** - User access management, authentication and encrypted communications protocols. The users can be organized into groups (roles).
3. **Workflow** - The workflow layer is the most important for carrying out drug development. The individual tools are organized and interconnected in a standardized way. Emerging paradigm for running complex, interrelated sets of software tools involve packaging software using Linux container technologies, such as Docker, and then orchestrating pipelines using domain-specific workflow language such CWL (Common Workflow Language).
4. **Back-end** - Certain tools included in the SILICOFCM platform are not intended to be used as workflows directly by users and developers, but either by standardized RESTful APIs or by user interfaces provided by the platform. That is also valid for the access to the research data available in the platform.
5. **Front-end** - A set of UIs employing underlying services.

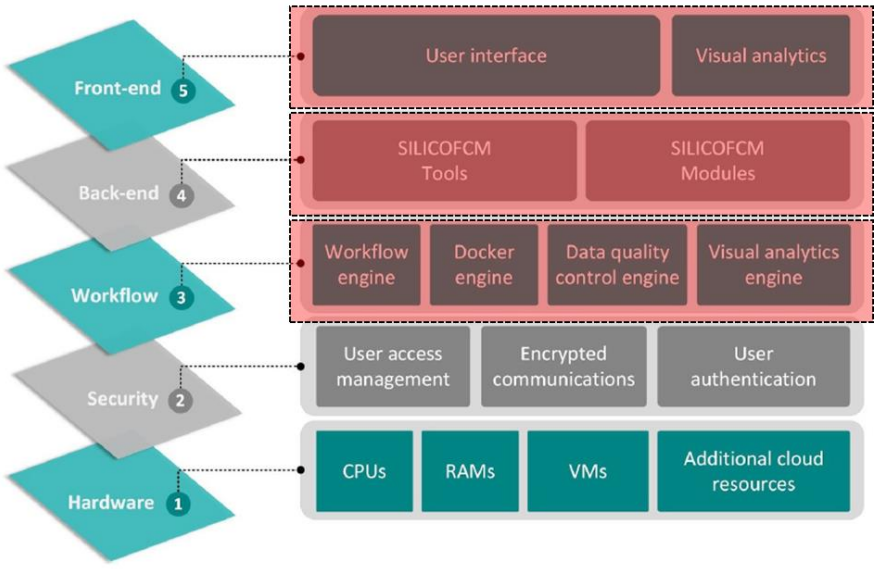



**Figure 3.** The architecture of the SILICOFCM platform.

## 3.2 Workflow management

Scientific workflow systems for use by biomedical researchers are based on an abstract representation of how a computation proceeds in the form of a directed graph, where each node represents a task to be executed and edges represent either data flow or execution dependencies between different tasks. There are numerous either open-source or proprietary systems such as Galaxy, Nextflow, TOIL, etc. Some of them also provide a visual front-end, allowing the user to build and modify complex applications with little or no programming expertise.

In order to be practical, but also to promote open-science principles, all data, analytical tools and methods should be *findable, accessible, interoperable and reusable* (FAIR principles). The FAIR principles serve as a guideline for data producers and researchers to be interoperable as much as possible[23]. The individual tools are organized and interconnected in a standardized way. This automatically implies packaging software using Linux container technologies, such as Docker or Singularity, and then orchestrating workflows and pipelines using domain-specific workflow language such WDL (Workflow Description Language) and CWL (Common Workflow Language).

Public clouds also provide batch processing capabilities (AWS Batch, EKS and ECS, etc.) that automatically provision the optimal quantity and type of compute resources based on the volume and specific resource requirements of the batch jobs submitted, thereby significantly facilitating analysis at scale. Figure 4[23] illustrates integration of data producers, consumers, and repositories via a cloud-based platform that supports FAIR principles.

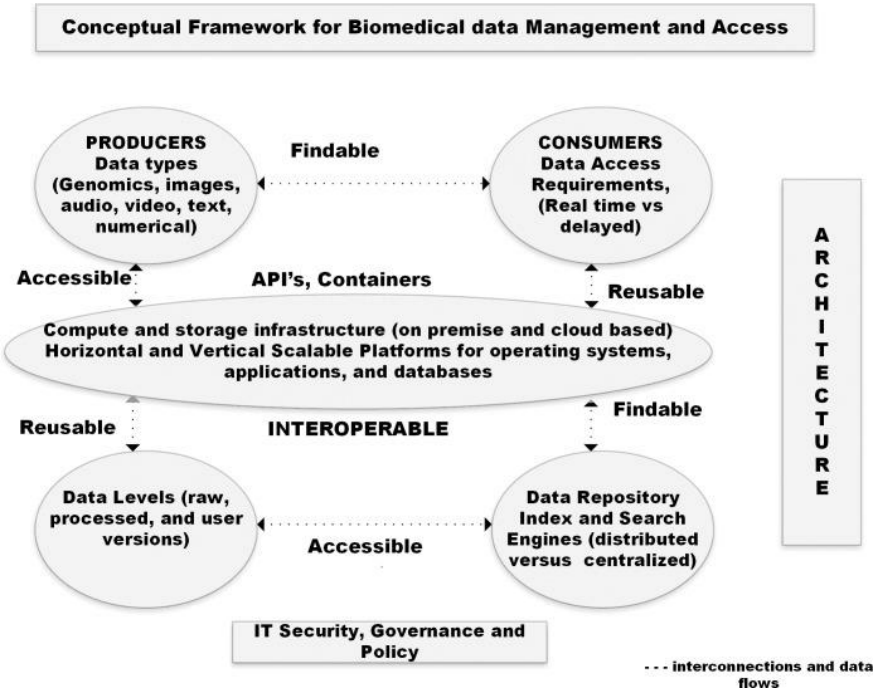



**Figure 4.** FAIR principles in an example.

The integration of genotype, phenotype, and clinical data is very important for biomedical research. The complete cloud platforms nowadays provide an environment for establishing an end-to-end

[23] Navale, V., & Bourne, P. E. (2018). Cloud computing applications for biomedical science: A perspective. PLoS computational biology, 14(6), e1006144.

pipeline for data acquisition, storage, and analysis. For example, one of the partners in the SILICOFCM project, Seven Bridges Genomics (SBG) offers both genomics SaaS and PaaS and employs AWS as backend. SBG Platform also enables researchers to collaborate on the analysis of large cancer genomics data sets in a reproducible and scalable manner. They base all their workflow management on a standardized Common Work-Flow language (CWL) to facilitate developers, analysts, and biologists to deploy, customize, and run reproducible analysis methods. Users may choose from over 500 tools and workflows covering many aspects of genomics data processing to apply for example to TCGA dataset or their own datasets.

For the sake of compatibility and to stay on track with modern trends and portability requirements, the SILICOFCM platform opted for CWL to provide all the workflows, including genomics, but also single-scale and multi-scale mechanics, CFD, electrodynamics, post-processing and many others. A specially-dedicated API dubbed Functional Engine Server[24] (FES-API) has been developed within SILICOFCM, capable of handling the entire lifecycle of multiple workflows simultaneously, as shown in Figure 5.

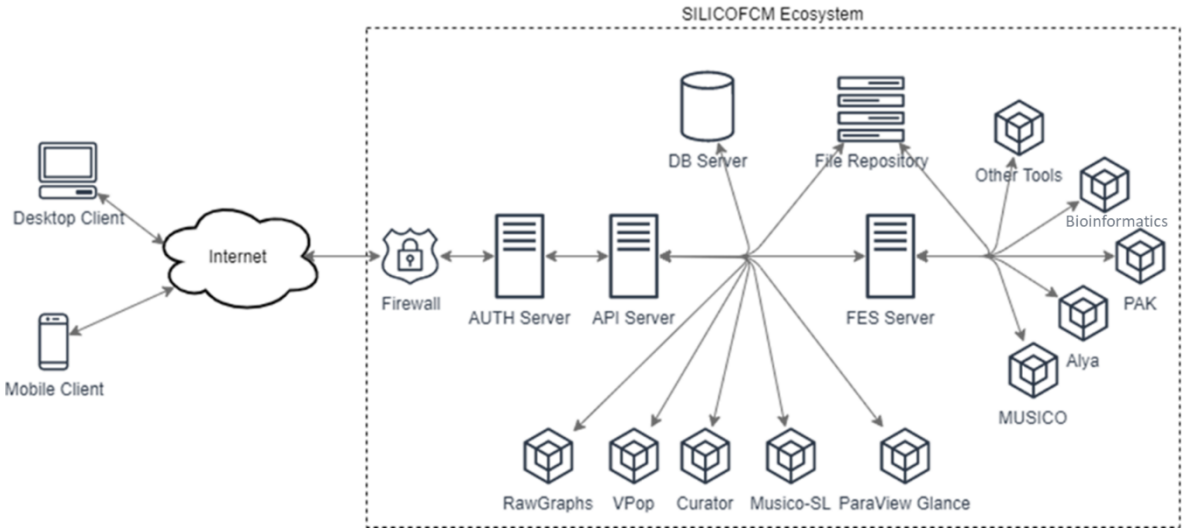



**Figure 5.** The role of the Functional Engine Server (FES-API) in the SILICOFCM platform.

[24] https://github.com/Andreja28/FES-API

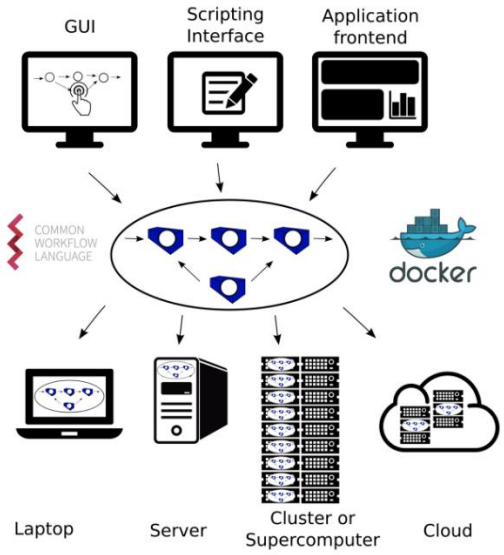



**Figure 6.** The execution platforms available for running Common Workflow Language (CWL) compatible workflows.

As shown in Figure 6, a number of CWL workflow executors can carry out the execution employing various execution environments, including desktop, server, HPC (High Performance Computing) clusters, as well as various public cloud providers. Unfortunately, the reference executor *cwltool*[25] is not capable of employing remote computing resources. However, TOIL, another workflow engine[26] is fully capable of running any CWL workflow in various computing environments, including public clouds.

One of the basic requirements posed by the SILICOFCM committee from the very beginning was to have the ability to deploy the entire platform (Figure 3) either on-premises or on any public cloud providing basic IaaS services. The main deployment (both staging and development) resides on premises of the University of Kragujevac Computing Centre. That primary installation is deployed on a capable Proxmox VE cluster, described in D7.2, with 10Gbps network and 20TB raw storage. However, despite the large capacity, on-premises deployment has certain, non-eligible, limitations. Firstly, the storage capacity could become a bottleneck when running large workflows, especially genomics. Secondly, despite the asynchronous capabilities of the FES-API server, capable of running multiple workflows started by multiple users, the scalability of such a system is somewhat limited, resource wise.

For all these reasons, the SILICOFCM deployment team made the feature complete SILICOFCM's twin available on AWS. In the next section, the specifics of AWS deployment will be described.

## 3.3 Running Drug Testing Workflows on AWS

The complete SILICOFCM platform has been deployed in AWS, employing standard, secure and well-established AWS services such as:

- EC2 for managing virtual instances,
- EBS for block storage,
- S3 for object storage, and

[25] https://github.com/common-workflow-language/cwltool
[26] https://toil.readthedocs.io/en/latest/

- Route 53 for domain name service (DNS).

As stated in the previous section, the major motivation points for porting at least the FES-API server to AWS are:

- Overcoming the potential issue of the insufficient storage by employing S3 object storage. The S3 bucket is available on the mount point in FES-API EC2 instance, providing "infinite" storage capacity for both running and completed workflows.
- Large potential to serve multiple users with multiple running workflows at the same time by employing multiple EC2 computing instances.

As stated above, FES-API is capable of using official CWL engine *cwltool*, but also TOIL workflow executor. The latter has better capabilities to interface to AWS API directly especially S3 and EC2 and handle resource requests automatically. The architecture of the TOIL engine is shown in Figure 7. The only instance to be manually deployed is the *Leader*. In its auto-scale mode, the *Node provisioner* launches an appropriate EC2 instance if necessary and joins it to the Apache Mesos[27] cluster. As soon as an instance joins a cluster, it is ready to execute the workflow requested by FES-API.

The scalability is not only provided when the load is increasing. One of the best features of TOIL is that an EC2 instance automatically switches off upon completing the requested workflow and transferring the results into S3 bucket. This feature contributes to infrastructure cost savings significantly.

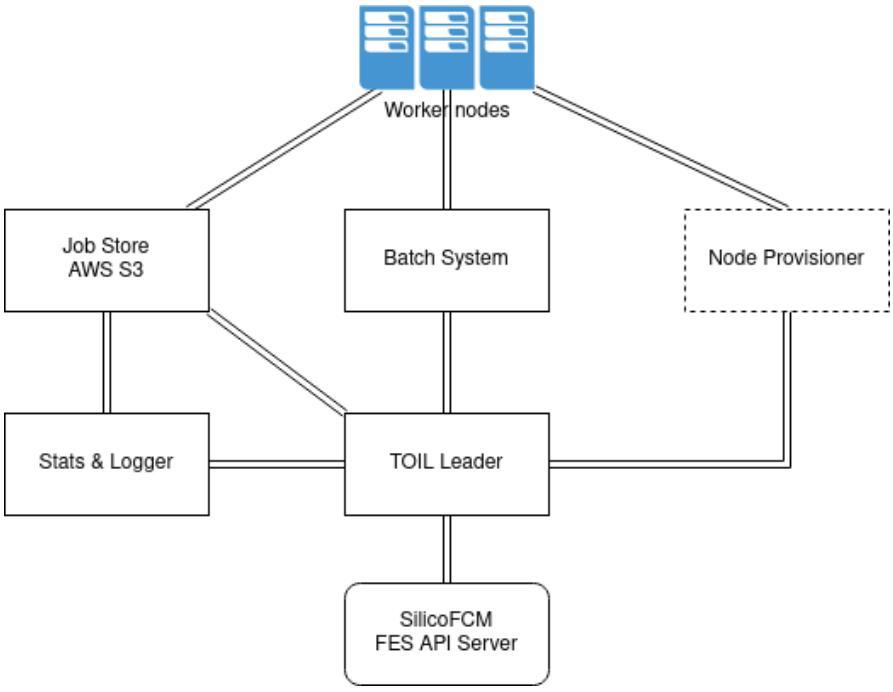



**Figure 7.** The architecture of the TOIL open-source workflow engine.

[27] http://mesos.apache.org/

# 4 SILICOFCM Workflow for drug testing

Integration of the computational tools from WP4, WP5 and WP6 is set for simulation of the effect of various drugs on impearled cardiac function caused various cardiomyopathies. This approach is suitable for accelerating new drug development or for testing drugs used in clinical practice. The multiple modules are implemented in the SILICOFCM computational cloud platform. This computational platform includes the following components:

(i) Client application Layer available through the web (access layer using a web browser),
(ii) Cloud server computational Layer (data processing layer and compute engines including Restful services' providers),
(iii) Data and Models management Layer,
(iv) Cloud data repository Layer.

In addition, a variety of experimental data and a system of interconnected computational tools integrated in the cloud platform enable quantitative assessment of the effects of drugs at different length and time scales, suitable for drug testing.

## 4.1 Concept and architecture

The computational tools developed during the SILICOFCM project or software developed prior the beginning of the SILICOFCM project by several project partners are integrated into the SILICOFCM platform. This platform contains multiple modules that interconnect the experiments from molecular interactions to whole heart physiological function. It is an outstanding tool for supporting the drug testing. The workflow through the system is shown in Figure 8 and the pathway through the modules connecting the experiments and supporting databases specific for each drug action is described below.

The workflow consists of three drug-testing pathways depending on the principal action of drug with different tools integrated. Specifically, each of the pathways should follow the effects of principal action of a selected drug at different scales starting from molecular interaction and their regulation to the effects on function at the organ level. We have developed three characteristic pathways of drug flow:

(i) for drugs acting at the level of contractile proteins;
(ii) at the level of regulation of transient intracellular calcium concentration;
(iii) at the level tissue remodeling and/or by modulation of blood vessel elasticity, i.e. resistance to blood flow and cardiac output.

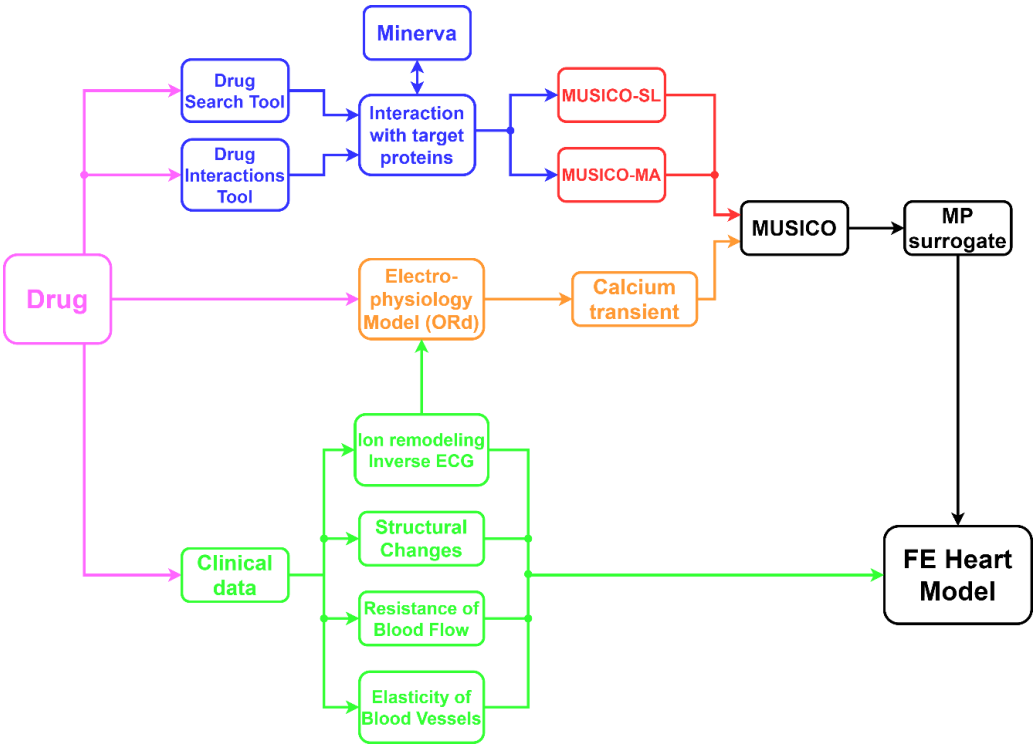



**Figure 8.** Diagram of drug testing workflow in SILICOFCM.

In order to run these workflows, it is necessary to have information about effect of drug, including the dose response. The experimental evidence of the system response to drug can be collected at different length scales and projected by using computational tools to assess the effect on higher, physiologically relevant scales. In the report D8.2, Section 3, we show the case studies for a few selected drugs acting on three major workflow pathways shown in Figure 8. These are representative examples of workflow for drug testing designed for specific Case studies collectively demonstrating robustness of the approach, and this small set of studies can be expanded to many other drugs in the future. Further details about the drug interaction tool and the drug search tool are given in sections 3 and 4 in D8.3 "Interface for drug testing".

### 4.1.1 Drugs that modulate [$Ca^{2+}$] transients

HCM is usually considered a disease of the sarcomere, because in most cases it is caused by mutations of one or more of the sarcomeric proteins; however, in a significant number of cases changes in cardiomyocyte electrophysiology and $Ca^{2+}$ homeostasis has been reported to be part of all human HCM disease phenotypes[28]. These changes are not directly related to the sarcomeric mutations; rather they occur as adverse remodelling due to disease-associated alterations of cardiomyocyte signaling. When compared with control cells, human HCM cardiomyocytes showed prolonged action potentials, frequent afterdepolarizations, slower $Ca^{2+}$ transients and elevated diastolic $Ca^{2+}$ concentration, largely determined by $Na^{+}$ and $Ca^{2+}$ cardiomyocyte overload[28]. These electro-mechanical abnormalities may be reversed by negative inotropic drugs able to decrease the Na+ and Ca2+ overload with beneficial effects on diastolic function and cellular arrhythmias.

[28] Coppini, R., Ferrantini, C., Yao, L., Fan, P., Del Lungo, M., Stillitano, F., ... & Mugelli, A. (2013). Late sodium current inhibition reverses electromechanical dysfunction in human hypertrophic cardiomyopathy. Circulation, 127(5), 575-584.

### 4.1.2 Drugs that affect changes in kinetic parameters

HCM and DCM are considered as serious diseases of the sarcomere, because, in most cases, muscle contractility is compromised by sarcomere protein mutations or changes in composition of the protein isoforms. For treating the cardiomyopathies caused by changes in kinetics and structural features in the sarcomere proteins there are several drugs or drugs under development to enhance muscle function, acting at the level of protein-protein interaction. Typically, drug action modulates or compensates for cardiac function impairment affected by specific mutilations or altered function at protein level that are usually accompanied with structural changes such as thickening or thinning ventricle walls. These abnormalities at protein level can be modulated by inotropic drugs able to increase or decrease cardiac muscle contractility with beneficial effects on systolic and diastolic function in HCM and DCM cardiomyopathies.

### 4.1.3 Drugs that affect changes in macroscopic parameters

About half of cardiomyopathies are caused by genetic malformations with mutations in sarcomeric proteins[29]. In addition to significant changes at the level of molecular mechanisms within cardiomyocytes, significant changes are also observed at the macroscopic level in terms of changes in blood pressure, left ventricular mass index, wall thickness, left ventricular diameter, left ventricular volume, fractional shortening, and ejection fraction. Change in these parameters induce many other physiologically important features and finally on health status of suffering patient. Many drugs are created to counteract these changes by reducing wall thickness, increasing left ventricular volume, or increasing ejection fraction.

[29] Vikhorev, P. G., & Vikhoreva, N. N. (2018). Cardiomyopathies and related changes in contractility of human heart muscle. International journal of molecular sciences, 19(8), 2234.

# 5 Drug database

## 5.1 Use of Minerva Hypertrophic Cardiomyopathy (HCM) map in the analysis of key intracellular signaling pathways

Minerva HCM map[30] is a is a detailed map of signaling pathways in the Hypertrophic Cardiomyopathy model of cardiomyocytes. It was created using literature data from (at this moment) a total of 102 scientific publications. The hcm map presents the most important factors (enzymatic proteins and structural cytoskeletal proteins) that define the basic molecular mechanisms within cardiomyocytes. The cardiomyocyte cell is represented by cellular compartments: Contractile apparatus, sarcoplasmic reticulum, mitochondria, cytosol, thin and thick filament systems. All cellular compartments are connected by signaling molecular mechanisms. Also, a signaling connection was made with surrounding cell types, such as cardiac fibroblast, skeletal muscle, adipocyte, and vascular endothelial cell. Interactions in the HCM map are marked with standardized features:

- → for cascade activation and
- ⊢ for cascade inhibition or inactivation.

The intuitive interactive HCM map enables the search for possible cascading interactions of individual factors with other factors. For example, if we know that a certain drug affects one of the protein factors, which is presented in the map, and if we know that a given drug activates a given factor, then marking a given factor opens a cascade of further interactions with other factors with information about possible cascade activation or inhibition. By monitoring a range of interactions, a distant indirect interaction of a given drug with parts within the cell can be predicted with a estimation of the physiological response.

The Minerva platform is connected to the ChEMBL drug database. Entering a specific drug of interest in the search field provides an answer about the possible interactions of a given drug with protein factors within the map. Further analysis of possible signaling pathways leads to a specific effect of the drug on the physiological parameters of the cell and further sarcomere and the whole heart.

### 5.1.1 Drug interaction

Literature data have indicated a number of drugs used in the treatment of cardiomyopathy, as well as heart failure. One of the most effective drugs today is Entresto® (Novartis, Switzerland). Entresto contains a combination of sacubitril and valsartan. It is used in certain people with chronic heart failure and is usually given together with other heart medications. This medicine helps lower the risk of needing to be hospitalized when symptoms get worse and helps lower the risk of death from heart failure. Two drugs in Entresto® synergistically reduce left ventricular remodelling and reduce cardiomyocyte death. Entresto® is also used to treat heart failure in children who are at least 1 year old.

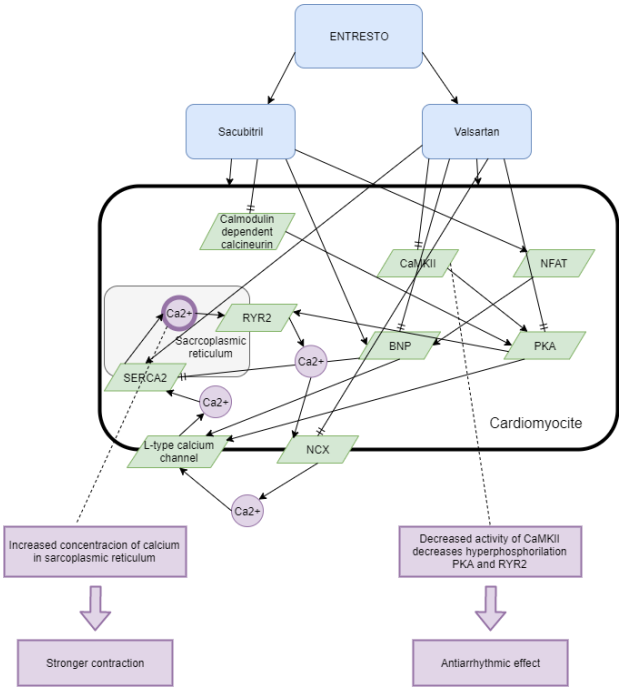
Sacubitril is a blood pressure medicine. It works by increasing the levels of certain proteins in the body that can dilate (widen) blood vessels. This helps lower blood pressure by reducing sodium levels.

[30] https://silicofcm-test.bioirc.ac.rs/minerva/

Sacubitril is a neprilysn (NEP) inhibitor prodrug with natriuretic activity. Upon administration, sacubitril is metabolized by esterases to its active metabolite, sacubitrilat, which inhibits NEP, a neutral endopeptidase that cleaves natriuretic peptides such as atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and c-type natriuretic peptide (CNP), as well as certain vasoconstricting peptides including as angiotensin I and II, and endothelin-1.

Valsartan is an angiotensin II receptor blocker (sometimes called an ARB), which selectively bind to angiotensin receptor 1 (AT1). It prevents angiotensin II from binding and exerting its hypertensive effects. These include vasoconstriction, stimulation and synthesis of aldosterone and ADH (antidiuretic hormone), cardiac stimulation, and renal reabsorption of sodium among others. Overall, valsartan's physiologic effects lead to reduced blood pressure, lower aldosterone levels, reduced cardiac activity, and increased excretion of sodium. Valsartan also affects the renin-angiotensin aldosterone system (RAAS), which plays an important role in hemostasis and regulation of kidney, vascular, and cardiac functions.

The data indicate the molecular mechanisms of the drug Entresto®. Based on these data, we used the Minerva platform to target specific cascade mechanisms and created a more detailed map of only a specific segment of the effect of this drug (Figure 9). Analysis of literature data and Minerva-ChEMBL connections indicated the most significant mechanisms of the cardiomyocyte drug effect. Also, the heart model responds to given inputs regarding the applied drug dose and physiological response via the Minerva platform. For example, 100 mg of the drug has been shown not to significantly alter Diastolic blood pressure (DBP). However, a dose of 200 mg reduces DBP by 2.97 mmHg, while a dose of 400 mg by 2.70 mmHg, resp[31]. Another example is that the use of 200 mg of the drug in patients with ejection fraction of ≤40% significantly decreased left atrial size (average 4.6 mL)[32]. Entering such parameters significantly improves the heart model. A similar procedure can be applied to any drug of interest.




[31] Wehland, M., Simonsen, U., Buus, N. H., Krüger, M., & Grimm, D. (2020). An evaluation of the fixed-dose combination sacubitril/valsartan for the treatment of arterial hypertension. Expert opinion on pharmacotherapy, 21(10), 1133-1143.
[32] Hubers, S. A., & Brown, N. J. (2016). Combined angiotensin receptor antagonism and neprilysin inhibition. Circulation, 133(11), 1115-1124.

**Figure 9.** Entresto® influence on physiological parameters of cardiomyocytes.

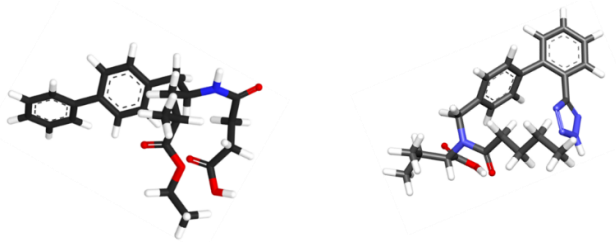
## 5.1.2 Drug interaction – molecular docking simulations & quantification

Using advanced molecular docking methods, it is possible to quantify and predict the interaction of any drug with any protein of interest. For example, the literature suggests that the Entresto® component of valsartan interacts primarily with the angiotensin I (Ang-I) receptor and with the angiotensin II (Ang-II) receptor. It is also known that this interaction is more significant with the Ang-I receptor. However, using the molecular docking methodology it is possible to quantify the given interactions and predict the probabilities of *ligand-protein* interactions.

Here are presented results of investigation and analysis of interactions between neprilysin and sacubitril, and human Angiotensin Receptor and Valsartan. Before molecular docking simulations are started, the binding sites of mention proteins are determined using POCASA. POCASA (POcket-CAvity Search Application) is an automatic program that implements the algorithm named Roll which can predict binding sites by detecting pockets and cavities of proteins of known 3D structure. Firstly, a 3D grid system is created and filled with atoms in the protein molecule. Second, a probe sphere is adapted to roll along the protein surface to generate a "probe surface" based on the inner border tracing algorithm in the image processing field. Then, the regions between the protein and probe surface or those surrounded by the protein surface are defined as pockets and cavities, respectively. In the case of Neprilysin, 19 binding sites are detected, while in the case of Angiotensin Receptor AT1,11 binding sites are predicted, and in the case of AT2 8 binding sites are predicted. The molecular docking simulations are carried out using the AutoDock 4.0 software. The three-dimensional crystal structures of Neprilysin and angiotensin receptor are obtained from the Protein Data Bank (PDB IDs: 6gid Neprilysin[33], 4yay Human Angiotensin Receptor AT1[34], and 5xjm Angiotensin Receptor AT2[35] respectively).

The preparation of protein for docking is carried out in the Discovery Studio 4.0 (BIOVIA Discovery Studio 2016). The co-crystallized ligand, water molecules, and co-factors are removed using this software. To add polar hydrogen atoms and to calculate Kollman charges the AutoDockTools (ADT) graphical user interface is used. In molecular docking simulations, the ligand is set as flexible, while the protein remained as a rigid structure. The bonds in ligand are set to be rotatable. The Lamarckian Genetic Algorithm (LGA) method is performed for protein-ligand flexible docking. The molecular docking simulation is done at a temperature of 298.15 K. Analysis of docking results and visualizations of linking positions are performed by using BIOVIA Discovery Studio.

Figure 10 presents structures of Sacubitril and Valsartan.



[33] Moss, S., Subramanian, V., & Acharya, K. R. (2018). High resolution crystal structure of substrate-free human neprilysin. Journal of structural biology, 204(1), 19-25.

[34] Zhang, H., Unal, H., Gati, C., Han, G. W., Liu, W., Zatsepin, N. A., ... & Cherezov, V. (2015). Structure of the angiotensin receptor revealed by serial femtosecond crystallography. Cell, 161(4), 833-844.

[35] Asada, H., Horita, S., Hirata, K., Shiroishi, M., Shiimura, Y., Iwanari, H., ... & Iwata, S. (2018). Crystal structure of the human angiotensin II type 2 receptor bound to an angiotensin II analog. Nature structural & molecular biology, 25(7), 570-576.

**Figure 10.** The structures of Sacubitril (left) and Valsartan (right)

By measuring the binding energies and many other parameters for each binding site, it is possible to predict the exact drug interaction with the factor presented e.g. in hcm map (Figure 11).

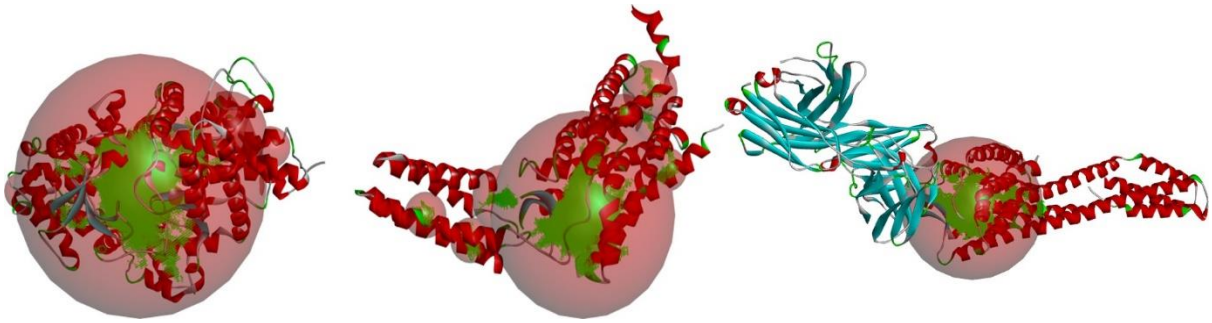


**Figure 11.** Representation of maps of bindings sites of neprilysin (left) and AT1 and AT2 (right)

This advanced molecular docking methods are currenlty out of the SILICOFCM platform, but it will be integrated until end of the project lifetime.

### 5.1.3 Future steps to improve the Minerva HCM map

After establishing the heart model, it is necessary to improve the Minerva HCM map and anticipate more possible interactions related to the type and dose of the drug in relation to the possible physiological responses. This will require focusing on the following tasks:

- Creating a more detailed map of signaling paths
- Linking the effect of the drug on changes in the cytoskeletal structure of cardiomyocytes
- Associating cytoskeletal changes with cell and sarcomere contractions
- Linking the influence of the drug on the gene expression of important parameters and
- *In silico* molecular docking prediction of protein-protein interactions.

In the framework of these tasks, special emphasis will be placed on protein-protein interactions and analysis of interactions with mutant forms of proteins, which are encoded by mutant forms of genes marked within *D4.1 Reference Graph Genome of Cardiomyopathy*. All of these tasks will be integrated during project lifetime.

## 5.2 Ventricular cardiomyocyte model

O'Hara-Rudy[36] model was used for human ventricular cardiomyocytes. This model contains the key currents (15 ionic currents) relevant in drug-induced arrhythmias.

$$I_{ion} = I_{CaL} + I_{Na} + I_{CaNa} + I_{CaK} + I_{Cab} + I_{Nab} + I_{Kb} + I_{Kr} + I_{Ks} + I_{K1} + I_{to} + I_{NaK} + I_{pCa} + I_{NaCa,i} + I_{NaCa,ss}$$

where the currents are L-type calcium current $I_{CaL}$; the fast and late sodium currents $I_{Na}$; the calcium sodium and calcium potassium currents $I_{CaNa}$ and $I_{CaK}$; the background calcium, sodium, and potassium currents $I_{Cab}$, $I_{Nab}$, and $I_{Kb}$; the rapid and slow delayed rectifier potassium currents $I_{Kr}$ and $I_{Ks}$; the inward

[36] O'Hara, T., Virág, L., Varró, A., & Rudy, Y. (2011). Simulation of the undiseased human cardiac ventricular action potential: model formulation and experimental validation. PLoS Comput Biol, 7(5), e1002061.

rectifier potassium current $I_{K1}$; the transient outward potassium current $I_{to}$; the sodium potassium pump current $I_{NaK}$; the sarcolemmal calcium pump current $I_{pCa}$; and the sodium calcium exchange currents $I_{NaCa,i}$ and $I_{NaCa,ss}$.

The O'Hara-Rudy model was applied to 3 different cell types - endocardial, midwall and epicardial cells. Figure 12 illustrates the single cell action potential of the O'Hara-Rudy model for endocardial, mid and epicardial human ventricular cardiomyocytes. We adopted the code of the original O'Hara-Rudy model[37] converted it to Fortran, and kept all the parameters as in the original model.

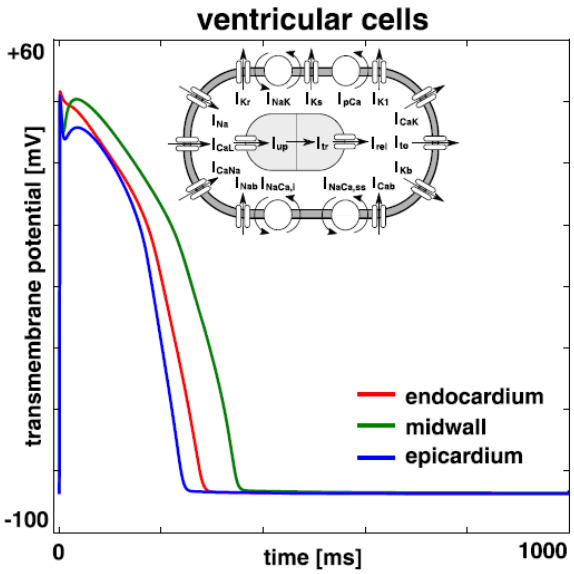



**Figure 12.** Single cell action potential for human ventricular cardiomyocytes. The ventricular cell model distinguishes between endocardial, midwall and epicardial cells and is based on the modified O'Hara-Rudy model with 15 ionic currents and 39 state variables.

There are several models in literature for the drug/ion-channel interaction studies[38]. In most of the cases, a drug affects cardiac ion-channel currents by direct binding. Blocking processes are connected to obstruction of the flow of ions through a channel pore. It can be done either by forming a physical obstacle, or by changing the conformation of the ion channel.
Some drugs such as pentamidine reduce whole-cell $I_{Kr}$ by interference with hERG expression and protein trafficking[39]. Drugs denoted as drug-blocks actually reduce the maximum conductance of an affected ion channel or transporter. In the modelling world it can be simulated with a scaling factor. This factor actually represents a function for the dose–response curve, describing the effect of a compound on the maximum current flowing through the target. Scaling factor b is reciprocally proportional to the drug concentration [D]

$$b = \frac{1}{1 + \left( \frac{[D]}{IC_{50}} \right)^{n}}$$

[37] O'Hara T, Virág L, Varró A, Rudy Y. ORd orginal human ventricular model http:\ignorespaces//rudylab.wustl.edu/research/cell/code/ AllCodes.html Accessed January 1, 2017; 2011.
[38] Brennan, T., Fink, M., & Rodriguez, B. (2009). Multiscale modelling of drug-induced effects on cardiac electrophysiological activity. European Journal of Pharmaceutical Sciences, 36(1), 62-77.
[39] Cordes, J. S., Sun, Z., Lloyd, D. B., Bradley, J. A., Opsahl, A. C., Tengowski, M. W., ... & Zhou, J. (2005). Pentamidine reduces hERG expression to prolong the QT interval. British journal of pharmacology, 145(1), 15-23.

where $IC_{50}$ is the drug concentration at which a 50% reduction of the peak current is observed, while *n* is the Hill coefficient of the dose–response curve. It is often assumed that n=1 which represents one drug molecule which is sufficient to block one ion channel[40].

## 5.2.1 Cardiac Fibroblast

During time, the number of fibroblasts in the heart changes with development, disease and aging[41]. In the healthy adult heart[42] fibroblasts comprise 30% to 70% of all the cells. Cardiac fibroblasts have been thought to play passive roles in the heart. Fibroblasts are well poised to actively regulate and modify cardiac function through their direct contacts with other cardiac cells and matrix.

In the embryonic heart, most fibroblasts are derived from epicardium (Figure 13 left section). In the adult heart, the dominant fraction of fibroblasts (~90%) in the RV and LV are of epicardial origin, while 64% of fibroblasts in the IVS derive from the endocardium (Figure 13 middle section). In response to pressure overload, the heart becomes hypertrophic and undergoes fibrosis. In response to pressure overload, both epicardium-derived and endocardium-derived fibroblast lineages proliferate and accumulate in interstitial and perivascular regions along with collagen I (black) (Figure 13 right section). The LV undergoes more fibrosis than the RV. Endocardium-derived fibroblasts participate in perivascular fibrosis in the IVS but not in the LV or RV. These two resident lineages account for approximately 95% of fibroblasts in the healthy and pressure-overloaded ventricles.

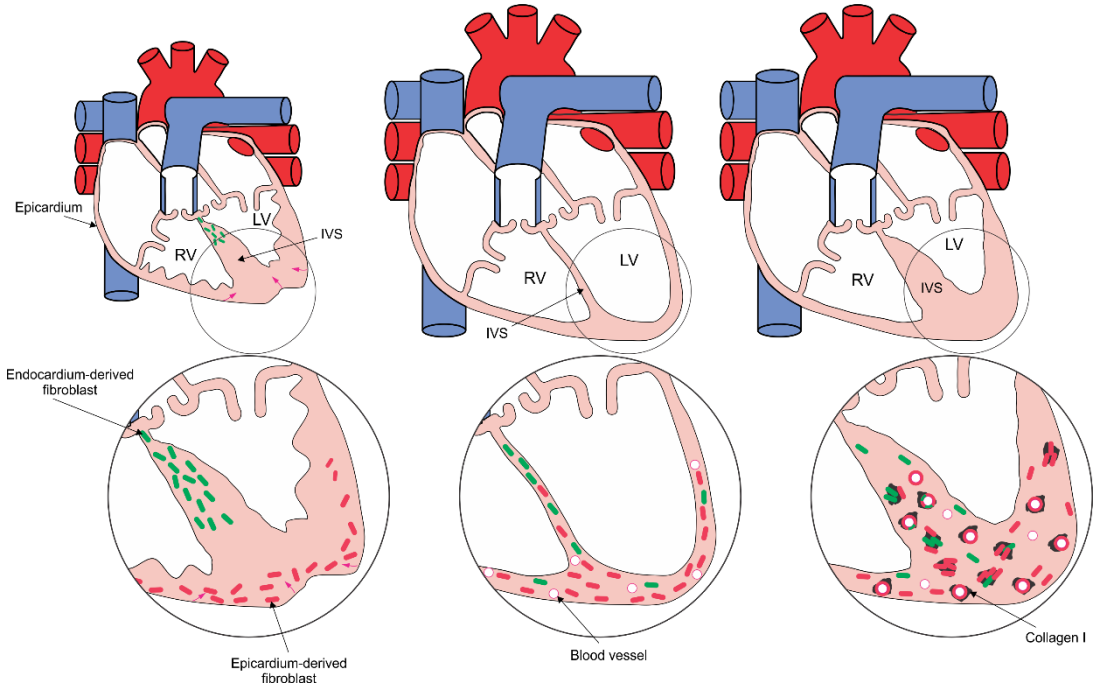



**Figure 13.** Cardiac fibroblasts in developing, adult and pressure overloaded hearts

[40] Mirams, G. R., Davies, M. R., Cui, Y., Kohl, P., & Noble, D. (2012). Application of cardiac electrophysiology simulations to pro-arrhythmic safety testing. British journal of pharmacology, 167(5), 932-945.
[41] Biernacka, A., & Frangogiannis, N. G. (2011). Aging and cardiac fibrosis. Aging and disease, 2(2), 158.
[42] Souders, C. A., Bowers, S. L., & Baudino, T. A. (2009). Cardiac fibroblast: the renaissance cell. Circulation research, 105(12), 1164-1176.

# 6 SILICOFCM modules for drug testing

## 6.1 MUSICO Tool

In order to trace the effect of the drugs on sarcomeric proteins, four modules from the MUSICO platform are used to precisely follow the implications of intracellular calcium and kinetic changes of small molecules (drugs) at multiple scales. MUSICO tools that are used for drug testing workflow are:

(1) MUSICO-SL for analysis of experiments in solution including, but not limited to: stopped- flow, titration and ATP-ase experiments. These simple and well-controlled experiments are an outstanding data source for developing the models of actin-myosin cycle, thin filament regulation by calcium. These computational tools are set for exploring the kinetic characteristics of the different isoforms of myosins and regulatory proteins (e.g. tropomyosin-C), or mutated sarcomeric proteins from data collected from an inexpensive experimental set up. In addition, this inexpensive experimental approach is an outstanding tool for testing the effect of different compounds on interactions between genetically altered and wild type (WT) sarcomeric proteins. The MUSICO-SL simulations provide the tool for the extraction of the key kinetics parameters including state transition rates in actin-myosin cycle and thin filament regulation by calcium that cannot be measured independently. In this report we also addressed challenges in translating these data to the systems of higher level of complexity, e.g. motility assays and muscle fibers.

(2) MUSICO-MA for the analysis of the motility assays. This, a bit more complex system, provides information, that cannot be accessed by experiments in solution and includes unloaded shortening velocities, loaded characteristics of sarcomeric protein interactions etc. These systems are widely used for development of new drugs by inexpensively testing the effect of large number of compounds on compromised sarcomeric protein interactions in (cardiac) disease caused by, for example genetic mutations. There are two kinds of motility assays: the first follows the movement of actin filament over lawn of myosin molecules attached on the cover slip, and the second that follows movement of actin filament over myosin filament attached to the cover slip. Both systems are currently under development under auspices of MUSICO-MA. In this report we show the analysis of motility assay of the second kind implemented in MUSICO-MA to assess the kinetic parameters of myosin binding protein C (MyBP-C). We also explored translation of the kinetic data to MUSICO simulations in muscle fibers and muscle tissue in quantifying the effect of MyBP-C mutations on compromised cardiac muscle function in hypertrophic cardiomyopathies.

(3) MUSICO for simulation of physiologically relevant cardiac contractions in muscle fibers, cells and tissues. We have developed the following new or refined modules:

- Six state actin myosin cycle including parked state,
- Kinetic model of thin filament regulation designed for calcium transient input necessary for simulations of physiologically relevant cardiac twitch contractions,
- Model of penetrance of myosin isoforms for simulations of the observed behavior of muscle fibers from patients with cardiomyopathies,
- Sarcomere geometry including thin filaments of variable length,
- The modules including structural sarcomeric proteins titin and MyBP-C. Kinetics of MyBP-C to actin filament translated for motility assay studies are also included in the MyBP-C model,

- The muscle fiber structural model also includes series elastic components necessary for simulations of twitches in cardiac trabeculae,
- The development of a module for mutational penetrance of troponin C (TnC) in transgenic trabeculae.

(4) MP Surrogate model: Mijailovich-Prodanovic (MP) surrogate model mimics simulations from 3D explicit stochastic model for computationally effective calculations with small memory requirements and fast execution time. It is a sliding filament model based on a solution of Ordinary Differential equations (ODE) and includes three kinetic processes of sarcomeric proteins interactions: (i) Ca2+ binding to troponin, that together with tropomyosin, forms the thin-filament regulatory unit (RU) and regulates availability of actin sites for myosin binding by switching between blocked and open RU states; (ii), myosin binding to actin where RUs are in open state, i.e., transition from detached to attached crossbridges in pre-power stroke state; and (iii) the transition from pre- to post-power stroke state. MP surrogate model also includes the overlap between actin and crossbridge populated region along myosin filament as a function of current sarcomere length, new serial elasticity, number of attached crossbridges regulated by calcium concentration and included an additional step in calcium activation of thin filament. MP surrogate model is essential for coupling MUSICO with the finite element (FE) simulations at organ level, for example whole heart.

## 6.2 PAK Solver Tool

PAK Solver Tool uses finite element method to calculate fluid-structure interaction to calculate blood flow velocity, pressure and shear stress in the fluid domain and for solid domain deformation and stress distrubution. Two different models are used on the SILICOFCM platform for PAK solver. Parametric model of the left ventricle runs quickly and get basic P-V diagrams to compare with the clinical measurement for the specific patient. A full coupled 3D model with real geometry from medical images of the particular patient represent the second model which take several hours of running on the cloud platform.

The P-V diagram plots volume along the X-axis and pressure on the Y-axis. The area of the loop is equal to the stroke volume, which refers to the amount of blood pumped out of the left ventricle in one cardiac cycle. The effects of isolated changes in preload are best demonstrated on the pressure-volume (P-V) diagram, which relates ventricular volume to the pressure inside the ventricle throughout the cardiac cycle. The maximum right point on the diagram is denoted as the end-diastolic volume (EDV), while the minimum left point as the end-systolic volume (ESV). Also, as EDV increases, the proportion of blood ejected by the heart increases slightly; this is the ejection fraction (EF) calculated by the equation: (EDV-ESV)/EDV. The reverse is also true. A decrease in preload will result in a leftward shift down the end-diastolic P-V line, decreasing EDV, stroke volume, and a slight decrease in ejection fraction.[43]

[43] Villars, P. S., Hamlin, S. K., Shaw, A. D., & Kanusky, J. T. (2004). Role of diastole in left ventricular function, I: Biochemical and biomechanical events. American Journal of Critical Care, 13(5), 394-403.

A variety of commonly used medications affected the cardiac function. Some of the first-line treatments for heart failure, myocardial ischemia and hypertension are described. Drugs that decrease preload and have influence on the cardiac PV diagrams are the following[44]:

- Angiotensin-converting enzyme (ACE) inhibitors – interrupts the renin-angiotensin-aldosterone system RAAS system. RAAS is a complex system responsible for regulating the body's blood pressure. The kidneys release an enzyme called renin in response to low blood volume, low salt (sodium) levels or high potassium levels.
- Angiotensin receptor blockers (ARBs) - interrupts the RAAS system.
- Nitrates – causes nitric oxide-induced vasodilation.
- Diuretics – promote the elimination of salt and water, resulting in a decreased overall intravascular volume.
- Calcium Channel Blockers – blocks calcium-induced vasoconstriction and decreases cardiac contractility.

## 6.3 Alya Solver Tool

This section is related to *In-silico* clinical trial using high performance computational modeling of a virtual human cardiac population to assess drug-induced arrhythmic risk. Also, it includes application to antimalarial and antibiotic drugs employed to treat COVID-19.

Drug-induced arrhythmias are a major health issue worldwide. The Covid-19 pandemic stressed the need for the creation of novel methodologies capable of providing urgent information about the cardiotoxic risk of employing two potential arrhythmic drugs as treatment. BSC embarked into the creation of a novel computational methodology to tackle the urgent needs arising with the pandemic. This novel methodology will be the basis for extending it to fulfill the SILICOFCM in-silico clinical trial requirements.

Currently, there are no predictors that can provide critical a-priori information regarding the risks of certain patients with normal QTc intervals to suffer from QT-prolongation after the administrations of one or various potentially cardiotoxic drugs. Once again, the need for such predictors became obvious during the early days of the SarsCoV2 pandemic, when it was uncertain whether the uses of hydroxychloroquine (HCQ) and azithromycin (AZM) could be more harmful than beneficial. It has been widely observed that males and females present a different risk to drug-induced arrhythmias and QTc-intervals due to sex-specific hormones. Furthermore, function of ion channels is significantly modified by environmental conditions (i.e. hormones, electrolyte concentrations and pH), which have substantial effects on the electrical activity. In the case of Covid-19, hypokalemia has been a prevalent condition in patients. Often little to no clinical information about drug interactions exists for a large number of drugs; therefore, these interactions can be characterized according to the most logical biophysical assumptions: potentiation or addition.

A variety of computational methods have been an important component for the study and assessment of drug-induced arrhythmias. However, full biventricular anatomies at the population level have never been employed.

[44] Sheth, P. J., Danton, G. H., Siegel, Y., Kardon, R. E., Infante Jr, J. C., Ghersin, E., & Fishman, J. E. (2015). Cardiac physiology for radiologists: Review of relevant physiology for interpretation of cardiac MR imaging and CT. RadioGraphics, 35(5), 1335-1351.

The first primordial objective of the work was to create a normal virtual population. The main hypotheses tested to achieve this aim were:

1. Gender-specific ion channel phenotypes are required to characterise gender-associated risk.
2. A diverse phenotypic population can be achieved by an array of computational heart models that incorporate the spectrum of variability on ion channel expressions quantified experimentally (as interquartile values of conductance magnitudes) and applied to the full biventricular anatomy.

The hypotheses regarding the drug administrations were:

1. Drug plasma concentrations and IC50 values (half-maximal inhibitory concentrations) reported in the literature (from a variety of experimental assays) have the same approximate effect on the virtual human population.
2. The two drugs administered in combination have an additive effect.
3. Arrhythmic risk can be assessed after a stress test on the virtual population. Indications of arrhythmic risk include conduction blocks, spontaneous ventricular tachycardia or asynchronous activation.

These four main hypotheses represent a gigantic leap from existing experimental single cardiomyocyte data to full heart physiology. The urgency for the questions posed clinically encouraged BSC researchers to assess them. The full pipeline is shown in Figure 14.

Our definition of the normal molecular expressions in the derived population was based on the characterizations of human cardiomyocytes by fitting their action potentials to an ion channel model. The conductances of five ion channels that are known to have higher influence the action potential duration (APD) were employed as the variable quantities that define the molecular expressions of ion channel kinetics within these virtual subjects. The cardiomyocyte mathematical model employed was the one published by O'Hara and Rudy with modifications from Dutta, and employed by the FDA as the basis for the CiPA initiative for proarrhythmic risk assessment with in-silico and in-vitro methods. Results of the application of this novel pipeline are under review for journal publication[45].

The computational clinical trial demonstrated the predictive capabilities of full heart simulations to reproduce the cardiotoxic effect after administration of one or more potentially arrhythmic drugs on a human virtual population. The comparison between clinical trials recently published in the literature and the virtual clinical trial show remarkable closeness. The data employed as input to the models were reported IC50 values and plasma concentration curves for each drug administered. The two drugs were assumed to have an additive effect.

The application of this computational framework will allow the preclinical testing of any new or existing drugs, potentially reducing animal experimentation and phase 1 trials, which in turn may result in a marked reduction of costs and safer and faster new drug use or repurposing of drugs in urgent situations like pandemics. Furthermore, this same pipeline is employed to assess drug effects on a population with hypertrophic cardiomyopathy and it is currently being integrated into the SILICOFCM platform.

[45] https://www.medrxiv.org/content/10.1101/2021.04.21.21255870v1

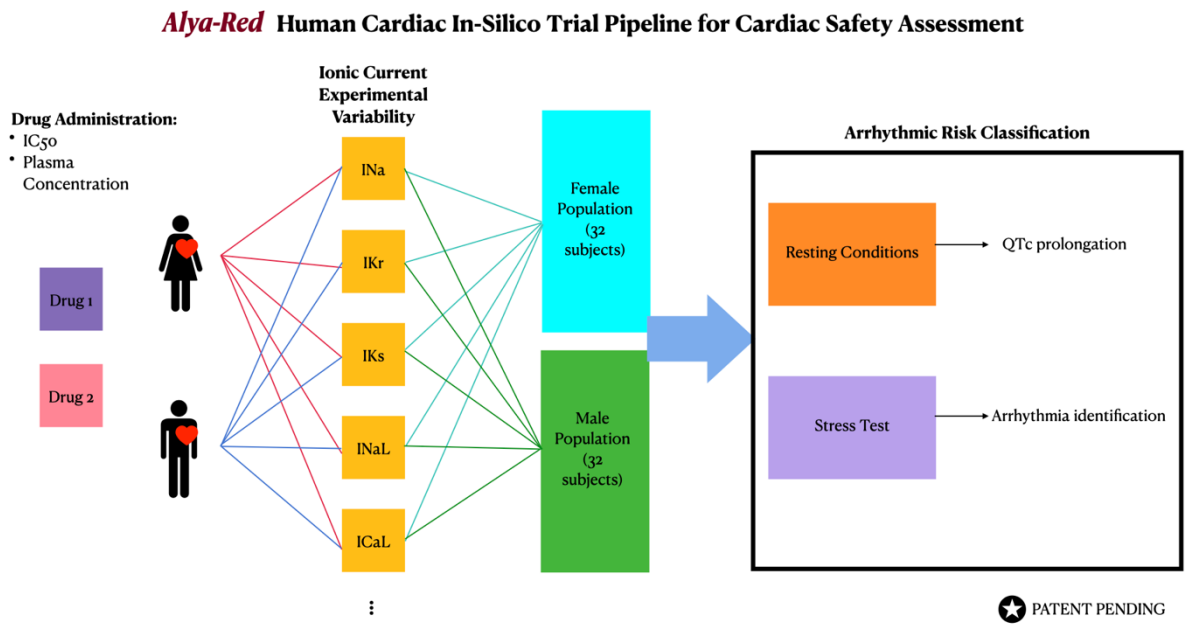



**Figure 14.** High performance computational pipeline for cardiac safety assessment using Alya-Red

## 6.4 Drug influence on the ECG simulation

Electrocardiogram (ECG) is the most widely utilized supplemental diagnostic tool for heart illness since it offers a wealth of cardiac beat information and clinical markers. ECG tests, which record the electrical activity of the heart, can aid in the early diagnosis of people with HCM.

The ECG is a tracing of projections of cardiac electrical potentials, called leads, on specific axes, depending on probes placement. Those leads represent a view of electrical activity of the heart from a particular angle across the body. The 12-Lead ECG has become a standard system used in clinical practice since the American Heart Association published the recommendation in 1954. It records signals from 10 electrodes respecting the following placement (Figure 15):

- V1: 4th intercostal space1 to the right of the sternum;
- V2: 4th intercostal space to the left of the sternum;
- V3: midway between V2 and V4;
- V4: 5th intercostal space at the midclavicular line;
- V5: anterior axillary line at the same level as V4;
- V6: midaxillary line at the same level as V4 and V5;

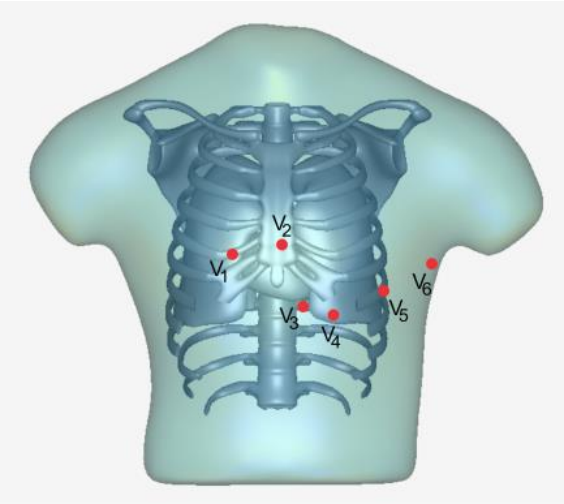



**Figure 15.** Six electrodes (V1-V6) positioned at the chest to model the precordial leads

Additionally, we have implemented classical approaches for solving the ECG inverse problem using the epicardial potential formulation. The studied methods are the family of Tikhonov methods and L regularization based methods[46,47,48].

It is a time-consuming process for doctors to make efficient and reliable diagnosis when confronted with tens of thousands of ECG recordings from various individuals. Furthermore, there are many noise interferences in the initially recorded ECG data, and the non-obvious potential deviation of unique nodes poses tremendous difficulty for cardiologists. Thanks to the fast development of computer-aided diagnosis technology, most commercial ECG devices now include an arrhythmia automated detection algorithm built in, however its high misdiagnosis rate is unacceptable[49].

A heartbeat is a single cycle in which the chambers of the heart relax and contract to pump blood, and each pulse has several waveforms. The electrical signal that travels through the heart chambers generates the ECG waves (atria and ventricles). A typical heartbeat and its waves (P, Q, R, S, T, and U) are depicted in Figure 16[50]. Inter-wave segments and intervals are also displayed.

The dataset contains between 4500 and 50,000 signal sample points. At the start, the ECG signal with inadequate length was filled with zero. In this way, information integrity was ensured to the greatest extent possible (as opposed to the irregularity of the waveform caused by the truncation of the single-heartbeat at an inappropriate position, the multi-heartbeat has information redundancy, so the position of the starting and ending points is no longer a limiting factor) and unnecessary computational overhead was reduced.

[46] Van Oosterom, A. (1999). The use of the spatial covariance in computing pericardial potentials. IEEE Transactions on biomedical engineering, 46(7), 778-787.
[47] A. Van Oosterom, (2003) Source models in inverse electrocardiography. Int J Bioelectromagn, 5:211–214.
[48] A. Van Oosterom (2010) The equivalent double layer: source models for repolarization. In Comprehensive Electrocardiology. Springer, pp. 227–246., 2010.
[49] Yu, S. N., & Chou, K. T. (2009). Selection of significant independent components for ECG beat classification. Expert Systems with Applications, 36(2), 2088-2096.
[50] ECG wave. [Online]. Available: http://lifeinthefastlane.com/ecg-library/basics/t-wave/

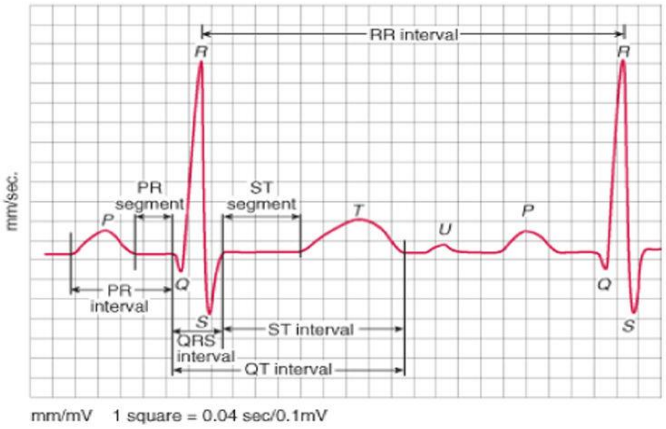



**Figure 16.** A typical heartbeat comprising P, Q, R, S, T, U waveforms and inter-wave segments and intervals

Traditional methods require implementing algorithms for extraction of features related to QRST complexes. In these cases, filtering is often accomplished by combining many bandpass filters, and it may efficiently reduce power frequency interference, remove electromyographic signals, and eliminate baseline drift. However, because the CNN can automatically minimize noise interference during the feature extraction process, and the data quality of the dataset utilized is greater, the influence of filtering on the model's final performance is not noticeable. In fact, we may choose whether to add a filtering operation based on the individual experimental setting or to isolate and filter signals with significant noise interference as well as multiple signals with high-quality needs.

Traditional methods use identification of the heartbeats that are simultaneously detected on all 12-leads. After segmenting the 12-lead ECG signals into individual heartbeats, extracted features from each heartbeat are represented as a feature vector used for classification. These features are usually:

- R-R interval
- P-wave duration
- QRS interval
- T-wave duration
- QRS morphology
- P and T wave morphology

Feature selection was also applied in order to identify the most significant features, after that classification process should be repeated with selected features.

The ECG dataset used in this study consists of 12-lead ECG signals from five sets of HCM patients:

1. Newcastle University (UNEW) consisted of 14 patients,
2. University Hospital Regensburg (UHREG) consisted of 12 patients,
3. Institute for Cardiovascular Diseases Vojvodina (ICVDV) consisted of 26 patients,
4. University of Florence (UNIFI) consisted of 9 patients,
5. Faculty of Medicine, University of Belgrade (FMBG) consisted of 41 patients.

Each patient was described by the medical expert with the established diagnosis. In total, the dataset consists of 102 HCM patients, and each patient has one or more ECG recordings in the dataset. The total number of ECG recordings in the dataset is 153. The UNEW is a coordinator of the clinical prospective study contained in WP3 and therefore responsible for data collection,

analysis, interpretation and presentation. At the time of the progression review meeting, participating clinical centres emailed data to the UNEW who collated data from all centres, screened for outliers and entered into a single database. The numbers indicated in the review comment i.e. 102 and 153 relate to patients assigned to an intervention (n=102) and total number of patients consented into the study (n=153) at the time for the progression review meeting. Data for all patients consented into the study will be further screened and included into final analysis once the data collection phase for clinical study is completed.

We used one-dimensional CNN that additionally had Inception and gated recurrent unit (GRU) modules. As some papers concluded that GRU modules are better than LSTM, a better solution is to add the GRU modules in the existing CNN instead of adding the LSTM modules[51]. The GRU module is presented in Figure 17b[51]. The GRU has the benefit of using a single gating unit to regulate both the forgetting factor choice and the update status decision. After the convolutional and inception layers, the GRU structure is utilized to identify the sequence properties of the P-wave, QRS wave, T-wave, and other wave groups. Inputting the updated results of the GRU layer's hidden layer cells to the hidden layer cells of the subsequent GRU layer can form a double-layer GRU, and inputting the double-layer GRU's last moment updated results into the full connection layer can complete the classification process via the Softmax function. The completely described network architecture is shown in Figure 17.

[51] Li, D., Wu, H., Zhao, J., Tao, Y., & Fu, J. (2020). Automatic Classification System of Arrhythmias Using 12-Lead ECGs with a Deep Neural Network Based on an Attention Mechanism. Symmetry, 12(11), 1827.

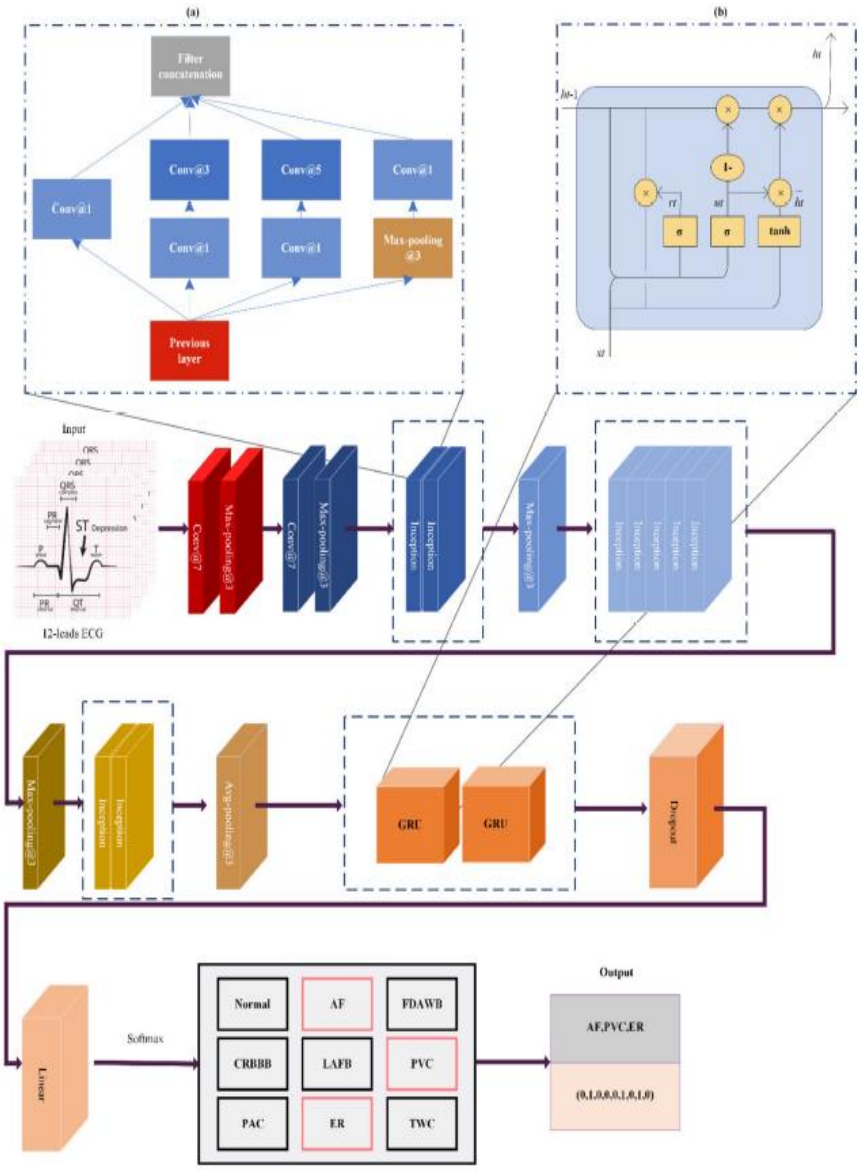



**Figure 17.** Architecture of the neural network. (a) Inception module. (b) GRU module

Also, in Figure 17a, the Inception module is presented. A varied size of convolution kernel is employed in each layer, which means that different sizes of receptive fields might obtain various feature representations. Furthermore, the 1x1 convolution kernel lowers the amount of parameter calculation bottlenecks produced by a large convolution kernel. Another key benefit of Inception is that each convolution module is made up of three layers: the convolution layer, the batch normalization layer, and the ReLU activation layer.

Currently, the integrated SILICOFCM AI model for Drug influence on the ECG simulation as well as comparison with ECG clinical measurement is in the testing and validation phase.

# 7 Regulatory bodies

## 7.1 EMA and FDA

Over the last years, the FDA has been very active in promoting computational modelling and simulations in medical device submissions by releasing guidelines and standards and providing consulting. Collaboration with European Competent Authorities is in place in the frame of the International Medical Device Regulators Forum (IMDRF) and of the Modelling and Simulation Working Party at the European Medicines Agency (EMA) level, which was created back in 2013.

In Europe, there is not a central regulatory body in charge of medical devices, such as EMA for medicinal products.

The European Commission funded the Avicenna Action to produce a research and technological development roadmap for *in silico* clinical trials. In 2018, the Commission reinforced its willingness to move forward by releasing a Communication to the European Parliament and other stakeholders on enabling the digital transformation of health and care in the Digital Single Market. On the same path, EMA published its Regulatory Science Strategy to 2025 on 31 March 2020, which is incorporating human and veterinary medicines. In this document, EMA issued a specific recommendation to optimize the capabilities in modelling, simulation and extrapolation, regarding the use of novel pre-clinical models - including those adhering to the 3Rs principle - and the development of guidance and standards on the use of AI in modelling and simulation for regulatory submissions.
Several initiatives are already planned and ongoing at the European level.
In the European Commission's Pharmaceutical strategy for Europe, under the topic "3.2. Enabling innovation and digital transformation", it is clearly written:
*The main source of evidence for the authorisation of innovative medicines should remain robust clinical trials with suitable comparators reflecting the standard of care in the EU. The full implementation of the Clinical Trials Regulation will put in place a harmonised, highly coordinated, robust and agile system for the assessment and oversight of clinical trials in the EU. It will improve transparency of information, independently of the outcome of the trials, to allow public scrutiny and will address new developments such as adaptive and complex trials, and* ***the use of in silico techniques and virtual approaches****. Experience with EU funded R&I projects with adaptive trials shows that research can initiate changes that can reduce costs and decrease development times*

The COVID-19 pandemic has boosted the need for the adoption of CM&S. However, for CM&S to be accepted as regulatory evidence there is an urgent need for internationally recognized standards. Indeed, in its Position paper "Proposal for a Regulation of the European Parliament and of the Council on the conduct of clinical trials with and supply of medicinal products for human use containing or consisting of genetically modified organisms intended to treat or prevent coronavirus disease", dated 8 July 8 2020, the European Economic and Social Committee (EESC) specifically stated that:

"*The EESC recommends that the Commission develop "Good Simulation Practices" so that industries have a definite regulatory framework within which to act when deploying computer modelling and simulation solutions in healthcare and in particular, in pre-clinical activity and in clinical trials, which is even more critical in times of public health emergency such as the COVID-19 pandemic.*

*As regards clinical trials, the Committee notes that there is a great deal of regulatory uncertainty surrounding the use of evidence derived from computer modelling and simulation (CM&S). These uncertainties prevent the uptake of CM&S solutions that could help prioritise and fast-track promising medicinal products and ensure that taxpayers only fund the safest and most effective treatments. The EESC therefore recommends that the Commission work to develop "Good Simulation Practices" to be*

*used when deploying CM&S solutions in healthcare and in particular in pre-clinical activity and in clinical trials*."

Indeed, guidelines and standards are essential to ensure credibility and reproducibility of CM&S, supporting their value and leading to acceptance by regulators.

Insofar, as previously described, the only available guidance document is the FDA-supported ASME V&V 40- 2018 "Assessing credibility of computational modelling through verification and validation: application to medical devices".

In Europe, the two most advanced initiatives towards the goal of standardization are the EU-STANDS4PM, a Horizon 2020-supported networking initiative aimed to develop universal standards, guidelines and recommendations for *in silico* methodologies relevant for personalized medicine, and the COMBINE (Computational Modelling in Biology Network) community, a network formed by the communities developing standards and formats to share computational models.

The process is not easy, since in order for standards to be officially adopted it will be necessary to have them approved by formal authorities like the International Organization for Standardization (ISO).

In view of facilitating and promoting standardisation, providing a summary of best practices on the use of CM&S in assessing the safety and efficacy of biomedical products, the Avicenna Alliance has recently established a dedicated Task Force to promote a consensus process for the definition of Good Simulation Practices (GSP), complementary to already existing GxP (Good Clinical Practice – GCP, Good Manufacturing Practice -GMP, Good Laboratory Practice – GLP).

In general, CM&S can be part of a regulatory submission in two ways:

i) when simulation is a medical device, used to support the clinical decision, that is "software as a medical device" and
ii) when the simulation results serve as supporting digital evidence in the regulatory dossier.

## 7.2 Communication with regulatory bodies

SILICOFCM project is part of the In Silico World community[52] which is a community devoted to accelerating the uptake of modelling and simulation technologies used for the development and regulatory assessment of medicines and medical devices, by lowering seven identified barriers: development, validation, accreditation, optimisation, exploitation, information, and training.
The GSP Task Force established by the Avicenna Alliance has recently met to define the list of its upcoming activities:

- a better definition of the scope, by actively seeking for the widest possible engagement with all organisations representing stakeholders (academia, industry, patients, regulators and payers)
- a wider dissemination of the work done so far
- a consensus process through the In Silico World Community of Practice[53]

The whole initiative is described in the In Silico World Community of Practice.

[52] https://insilico.world/
[53] https://insilico.world/community/good-simulation-practice-gsp-task-force/

First thing this grass root group produced was a collection of possible contexts of use for In Silico Trials that was published recently[54]. Now the group is working on the position paper on Good Simulation Practice[55].

So far through the In Silico World group we have had a meeting with various FDA officers, and a formal meeting with the Innovation Task Force of EMA.

SILICOFCM consortium has developed an automated platform, which is able to act as a decision support tool of the healthcare practitioners towards the treatment and treatment response of the patients. Based on its intended use the consortium plans to qualify this software system as a Medical Device based on the classification guidance of the software provided to the MDCG 2019-2011.

The traditional paradigm of medical device regulation was not designed for adaptive AI/ML technologies, which have the potential to adapt and optimize device performance in real-time to continuously improve healthcare for patients. Nevertheless, as part of the new regulations (MDR/ IVDR) the validation of Software as Medical Device will be separated into two parts.

It remains to be classified if the SILICOFCM platform will be a Medical Device or IVD. For this reason, a gap analysis shall be executed to determine the kind of data to be incorporated in order to allow the consortium the right product classification. Another point of view to be defined is whether the SILICOFCM could be classified also as companion diagnostic as will also provide information of drug pharmacokinetic, pharmacodynamic, and pharmacogenetic analysis.

In addition, SILICOFCM consortium puts continuous efforts towards regulatory pathways in ISCTs acting through the In Silico World community in communication with the regulatory bodies in order to utilize all relevant aspects and needs for approval of CM&S as part of biomedical products. It should be emphasised that SILICOFCM Advisory Board also actively participates and contributes to these directions.

Following the ASME VV-40-2018 standard, the In Silico World community and the SILICOFCM consortium as part of it, are taking actions towards credibility goals for the CM&S that will be achieved through careful planning and execution of model verification and validation activities. After a recently held meeting of *In Silico* projects, at the beginning of October 2021, and discussion about common interests in adoption of *in silico* trials, further steps towards verification and validation are undertaken. As one of drawbacks is lack of independent validation dataset collections, the *In Silico* projects aim to create a unique dataset by collecting different projects' results that are needed for achieving credible computational models. Activities associated with establishing the credibility of a computational model can be sub-divided into three categories: verification, validation and applicability. The objective of leveraging external patient-level data is to save time and bring new safe and effective technologies to market sooner.

All the SILICOFCM modules/tools underwent a preliminary validation (as a proof-of-concept) in order to be inserted in the "SILICOFCM today" pipeline for the first context of use. The different scenarios built in the SILICOFCM platform rely on sequences or combination of *in silico* tools and modules and constitute a preparatory step to the appropriate set-up of *in silico* trials. On the other hand, the adoption of a fully automatic *in silico* pipeline on the platform to be used for the second context of use requires a deeper validation and further exploiting the data collected during the SILICOFCM project is expected in the future ("SILICOFCM in the Future").

[54] https://ieeexplore.ieee.org/document/9462824
[55] https://insilicoworld.slack.com/archives/C01B4FRB7A8

To comply with the regulatory requirements, firstly the SILICOFCM platform has been developed based on the state-of-the-art standard IEC 62304 (EN 62304:2006) Medical Device Software – Software Life Cycle Processes. A software risk classification as per IEC 62304 shall be defined to clarify the documentation needed for software design verification and validation activities.

Additionally, the user interface characteristics will be defined by developing a Usability Engineering File based on the requirements of the IEC 62366-1:2015. The user interface risks will be determined and analysed to provide integrated solutions for a user safe and friendly environment.

As SaMD (Software as Medical Device), the SILICOFCM platform needs to be also validated/evaluated clinically based on the guidance document of MDCG 2020-1. Based on this European guidance document, the SILICOFCM platform will be evaluated based on:

a. its scientific validity through literature appraisal,
b. equivalent methodologies products and/or equivalent devices, (the SILICOFCM platform is an innovative product, therefore there are no equivalent medical devices, but this stage of evaluation will include similar technologies or devices with similar intended use),
c. clinical investigation during the project with clinical partners and finally,
d. risk-benefit analysis.

The SILICOFCM project targets the development of a safe technology as a medical device. For this reason, during the project a risk management process will be developed to cover requirements of ISO 14971:2019 for risk management related to medical devices including risk analysis, assessment, mitigation controls and risk re-evaluation. The risk-benefit analysis will be defined within the clinical evaluation/validation as a result of the risk management process, but also as a result of the clinical investigation process.

The Clinical Investigation provision will be considered for the SILICOFCM clinical validation as per Annex XV of MDR or Annex XIII of IVDR (depending always to the classification of the SaMD). Ethics provision and applications based on requirements of the national competent authorities will take place. The Clinical evaluation will be performed as a collective project between the clinical partners, the technical and regulatory partners by also expressing the absence of conflict of interest.

The Clinical Data (as mentioned above) are being collected from:

**Technical Performance:** the demonstration of the product ability to generate the intended output accurately, reliably and precisely, from the input data. The evidence to support the Technical Performance will be generated through verification and validation activities, e.g. unit-level, integration, and system testing or by generating new evidence through use of curated databases, curated registries, reference databases or use of previously collected patient data.

**Scientific Validity:** the product's output (e.g. concept, conclusion, calculations) based on the inputs and algorithms selected. The Scientific Validity of SILICOFCM will demonstrate that it corresponds to intended use. As such it will seek to establish that there are sound scientific principles underpinning the use of the SILICOFCM platform.

**Clinical Performance:** For the validation of the SILICOFCM platform, the consortium will demonstrate that the product has been tested for the intended use(s), target population(s), use condition(s), operating- and use environment(s) and with all intended user group(s) to provide the relevant Clinical Benefit to the patients. The Clinical Validation process will be considered for the latest version of the software. The consortium does not plan to demonstrate clinical performance studies for several software version releases.

**Final Analysis and Conclusion:** All the collected data shall be analysed into a Report's form to conclude the Risk -Benefit Ratio of the product and to ensure its safety towards the users and the end-users.

Schematic illustration of the clinical validation process is given as follows.

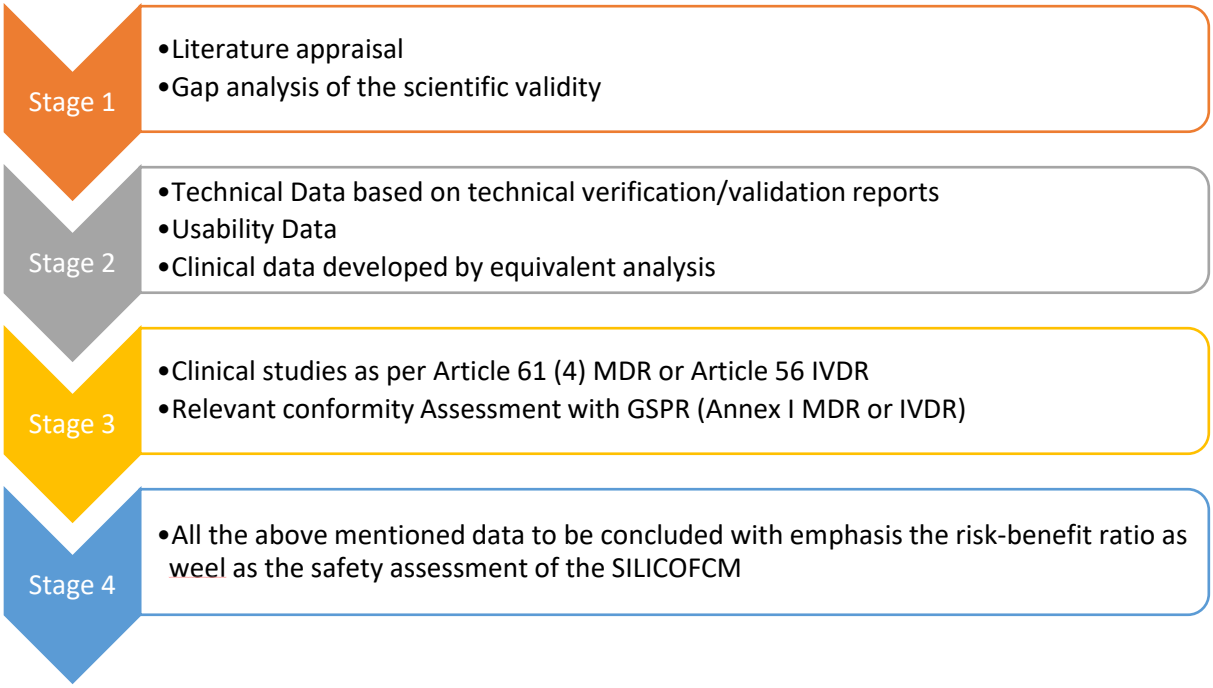



**Figure 18.** Schematic illustration of the clinical validation process.

Finally, taking into account that guidelines and standards are essential to ensure credibility and reproducibility of CM&S, supporting their value and leading to acceptance by regulators, *In Silico World* community that includes *in silico* projects, is working on Good Simulation Practices for the use of CM&S in the regulatory process of biomedical products. The synergy and common acting of *in silico* projects represent an added value in the regulatory pathways and in front of the regulatory bodies, enabling multilateral communication on a monthly basis.

## 7.3 Notified bodies

In Europe, the conformity assessments are performed by the Notified Bodies (NB), independent accredited entities appointed by the Member States that – as established by the EU MDR - are also responsible for monitoring NB activities. The EU MDR has introduced very strict rules and high standards for a NB to be requalified under the new Regulation; consequently, the number of currently approved NBs is still limited. Moreover, NBs have to face a great increase in activities linked to broader scope of the MDR, new classification rules and inclusion of some Class I devices under their intervention. Notified Bodies perform their assessment based on standards (ISO) and current regulation: lack of specific guidance for the assessment of *in silico* methods and lack of resources and specific know-how are important limiting factors.

# 8 Roadmap to the adoption of SILICOFCM platform

According to the exploitation plan and DoA, the aim of the SILICOFCM project is to develop an *in silico* clinical trial platform for the design and functional optimization of whole heart performance and monitoring effectiveness of pharmacological treatment. According to the assessment of the classification of the SILICOFCM platform and based on its context of use, it could be qualified as a Software as a Medical Device (SaMD) – FDA, a Medical Device Software (MDSW) – EMA.

## 8.1 Classification of Software as a Medical Device

IMDRF defines 'SaMD' as 'software intended to be used for one or more medical purposes that perform these purposes without being part of a hardware medical device'. The IMDRF provides clarification through notes, further supplemented by FDA guidance[56]. An important clarification is that the italicized term does not refer to the physical location from where the software is running but to the regulatory status of the software. Software can run on general-purpose IT equipment in 'the cloud' but also on the computing platform of a hardware medical device and still be SaMD. When the hardware medical device needs the software to achieve its intended medical purpose – for example because it drives the hardware or fulfils a purpose claimed for the hardware device – then the software is not SaMD but part of the medical device in the regulatory meaning of the term.

The IMDRF, FDA and EU use different definitions for 'medical device'. Despite these differences, the practical interpretation largely overlaps if one ignores the functional and CDS exemptions applied in the two regions. The definition of a medical device according to EMA and FDA is given in addition:

| | |
|---|---|
| EU | A 'medical device' means any instrument, apparatus, appliance, software, material or other article, whether used alone or in combination, including the software intended by its manufacturer to be used specifically for diagnostic and/or therapeutic purposes and necessary for its proper application, intended by the manufacturer to be used for human beings for the purpose of: diagnosis, prevention, monitoring, treatment or alleviation of disease, diagnosis, monitoring, treatment, alleviation of or compensation for an injury or handicap, investigation, replacement or modification of the anatomy or of a physiological process, control of conception, and which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means, but which may be assisted in its function by such means[57]. |
| USA | A 'medical device' means an instrument, apparatus, implement, machine, contrivance, implant, in vitro reagent, or other similar or related article, including a component part or accessory which is: recognized in the official National Formulary, or the United States Pharmacopoeia, or any supplement to them, intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man or other animals, or intended to affect the structure or any function of the body of man or other animals, and which does not achieve its primary intended purposes through chemical action within or on the body of man or other animals and which is not dependent upon being metabolized for the achievement of any of its primary intended purposes[58]. |

[56] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/clinical-decision-support-software
[57] Council Directive 93/42/EEC of 14 June 1993 concerning medical devices. OJ L 169, 12.7.1993. Amended by Directive 2007/47/EC of the European Parliament and of the Council of 5 September 2007. OJ L 247, 21.9.2007
[58] https://www.fda.gov/industry/regulated-products/medical-device-overview#What%20is%20a%20medical%20device

To summarize all the above mentioned, software application is classified as a medical device when it is developed for the medical purpose, particularly:

- diagnosing, curing, mitigating, treating, alleviating, compensating or preventing an injury or a disease;
- providing means and suggestions for mitigation of a disease;
- providing information for determining compatibility, detecting, diagnosing, monitoring or treating physiological conditions, states of health, illnesses or congenital deformities;
- aiding diagnosis, screening, monitoring, determination of predisposition; prognosis, prediction, determination of physiological status.

### 8.1.1 Classification

The new Medical Device Regulation (MDR)[59], published in April 2017 and which replaced the MDD (Medical Device Data) in May 2020, puts more emphasis on software. General-purpose software or software for lifestyle and well-being purposes is explicitly excluded from the MDR. Compared to the MDD, there is an additional classification rule (Rule 11) for software in the MDR, that covers other types of software, e.g. for clinical decision support.

**Rule 11** –

- Software intended to provide information which is used to take decisions with diagnosis or therapeutic purposes is classified as Class II a, except if such decisions have an impact that may cause:
  - death or an irreversible deterioration of a person's state of health, in which case it is in Class III; or
  - a serious deterioration of a person's state of health or a surgical intervention, in which case it is classified as Class II b.
- Software intended to monitor physiological processes is classified as Class II a, except if it is intended for monitoring of vital physiological parameters, where the nature of variations of those parameters is such that it could result in immediate danger to the patient, in which case it is classified as Class II b.
- All other software is classified as Class I.

According to the Rule 11 and the roadmap for adoption of the SILICOFCM cloud-based platform, it could be classified as SaMD, Class IIa. The two standards are under consideration:

- IEC 62304:2006 Medical device software - Software life cycle processes[60]. A harmonised standard for software design in medical products adopted by the European Union and the United States[61];
- (American Society of Mechanical Engineers) ASME V&V 40-2018: Assessing Credibility of Computational Modeling through Verification and Validation: Application to Medical Devices[62].

[59] https://decomplix.com/medical-software-mdr/
[60] https://www.iso.org/standard/38421.html
[61] https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfstandards/detail.cfm?standard__identification_no=38830
[62]https://www.asme.org/codes-standards/find-codes-standards/v-v-40-assessing-credibility-computational-modeling-verification-validation-application-medical-devices

## 8.2 Development plan

The aim of this section is to outline a step-wise regulatory development plan to use the SILICOFCM platform as a reliable source of information that could be a component of a regulatory submission.

Namely, the development plan will move from:

1. the utilization of simulations/*in silico* trials as a research tool/decision-support system in the functional optimization of whole heart performance and monitoring effectiveness of pharmacological treatment

to

2. modelling/*in silico* trials once the candidate/s treatment has been selected after the initial screening phase.

Practically, this step-wise approach defines two contexts of use that are consistent with the vision recently presented by Tina Morrison (FDA presentation: "Advancing *in silico* Medicine at the FDA: Perspectives on Simulation in Medical Devices", July 23, 2019).

In the "Future" path of the development process, there are a number of possible contexts whereby the use of CM&S can be envisioned, whereas in the "Today" designing path several SILICOFCM tools and databases for drug testing and functional optimization of whole heart performance are included.

All the SILICOFCM modules/tools underwent a preliminary validation (as a proof-of-concept) in order to be inserted in the "SILICOFCM today" pipeline for the first context of use. On the other hand, the adoption of a fully automatic *in silico* pipeline on the platform to be used for the second context of use requires a deeper validation and is expected in the future ("SILICOFCM in the Future"), further exploiting the data collected during the SILICOFCM project. A roadmap toward the regulatory acceptance can be outlined by defining all the issues to be faced step-by-step.

# *9* Deviation from the work plan

The presented document is revised and updated version of the deliverable D8.1 submitted on June 05th 2021. After the project review meeting which was held on July 05th 2021, the review team rejected the deliverable and gave the recommendations how it should be improved. The agreed deadline was the end of October 2021. The consortium took all the comments and recommendations from the review report into consideration and submitted the updated deliverable on November 2nd 2021.

# 10 Conclusions

The revised deliverable D8.1 “Workflow for drug testing” v2.0 corresponds to the work performed within Task 8.1 “Development workflow assistant for EMA/FDA approval” (M24-M36) of the SILICOFCM project. It results from the integrated approach for drug testing by deploying the cloud-based SILICOFCM platform, its tools and modules. The performed work is related to WP4, WP5 and WP6, and should be read together with D8.2 “Computational pipelines for drug testing”. In addition, the document is interlinked with D8.3 “Interface drug database”. The work that is being performed within the Task 8.1, as well as within the whole WP8, is a continuous process, and will be concluded in D8.4 “Development report tool”.

The deliverable D8.1 presents the workflows for computational modelling for basic drug testing for FCM. It includes the concept and architecture of and the scenarios for drug testing and effects on heart functions. It covers three different scenarios for effects of drugs on heart function: i) drugs that modulate Ca2+ transients, ii) drugs that affect changes in kinetic parameters, and iii) drugs that affect changes in macroscopic parameters. The integrated drug database describes the use of Minerva HCM map in the analysis of key intracellular signalling pathways. In addition, workflow for drug testing using MUSICO tool and PAK solver tool are presented. Drug influence on the ECG simulation and comparison with ECG clinical measurement are also described. Additionally, the approach for drug testing using Alya Solver tool is presented.

Finally, the regulatory context and notified bodies are analysed, together with the regulations for classification of software as a medical device. This resulted in the creation of the initial roadmap and development plan for the adoption of the SILICOFCM platform, following a stepwise approach: “SILICOFCM today” and “SILICOFCM tomorrow”. As this process is continuous, the finalized development plan and roadmap towards EMA and FDA will be included in D8.4 “Development report tool”.

# 11 References

The references are inserted as footnotes.