



The virtual assay software for human *in silico* drug trials to augment drug cardiac testing

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ABSTRACT

Prediction of drug effects on the heart still represents a challenge in drug development, given potential adverse outcomes. Computer modelling and simulations of the human heart offer a powerful technology to augment existing animal and clinical methodologies. Here we describe the translation process that led to the development and uptake of Virtual Assay, a user-friendly software to perform *in silico* drug trials in population of human cardiac models. Through this work, we contributed to the uptake of *in silico* modelling and simulations in industry and regulatory paradigms, and demonstrated accurate and mechanistic predictions of drug-induced cardiac pro-arrhythmic toxicity.

1. Introduction

We are all different, and this is the biggest challenge in the evaluation of the safety and efficacy of medical therapies. Gender, age, genetic background, disease and life style can all cause variations in the absorption, distribution, metabolism and excretion of drugs across individuals, and can also modulate biochemical and physiological effects of the drugs on the body [1,2].

A specific challenge in drug development is the early identification of adverse drug reactions, and specifically those affecting the heart. A recent review investigated 462 drugs withdrawn from the market between 1953 and 2013, and drug induced cardiotoxicity was one of the most prevalent reasons [3]. In addition, drug candidates often fail in pre-clinical phases or clinical trials due to adverse effects or limited efficacy, leading to significant loss of research time and funding. Therefore, an early detection of drug-induced cardiotoxicity can largely reduce the healthcare risk for patients, and can also relieve the economic burden in drug development.

Animal experiments are extensively used in the early examinations of drug safety and efficacy. These present important limitations [4–6]. Firstly, animals and humans exhibit significant pathophysiological differences, which compromise the clinical translation of animal-based predictions. Secondly, the large variability in genetic background, comorbidities and environmental factors present in the human population is ignored in animal experiments. The animals used are often

healthy, and from the same breed and sex. Therefore, human-based approaches that can capture key disease conditions and inter-subject variability are critical [7].

In this paper, we present our experience in translating a new human-based cardiac modelling and simulation approach for drug evaluation, from academic research to industry use. We specifically describe the foundations and development of the Virtual Assay software and its application for drug-induced pro-arrhythmic cardiotoxicity. The key advantages that human-based modelling and simulation bring are as follows. Firstly, their focus is on human pathophysiology, and therefore inter-species differences, important for clinical translation, are largely overcome. Secondly, they enable fast investigations of thousands of testing conditions, including diseases and genetic mutations, which are essential to mimic the variable drug responses in a large population. Thirdly, they allow integrative investigations of the mechanisms explaining the adverse outcomes. Fourthly, they can be implemented into the compound screening process to speed up the early discovery of cardiotoxicity with very low cost, building on ion channel screening.

2. Background, context and research innovation

2.1. Background and context

The technology presented in this paper is the product of a highly interdisciplinary blend of knowledge and expertise. Cellular physiology,

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genetics, cardiology, pharmacology, mathematics, engineering, physics and computer science have all been integrated over the years in the development of *in silico* tools for modelling and simulation of drug effects on human cardiac behaviour [7,8].

The main target of the simulations is the electrical signal generated by cardiac cells upon excitation, the action potential (AP), which is generated by ionic fluxes across the cellular membrane (ion currents), through large and complex proteins (ion channels). Simulations of the cardiac AP are conducted with biophysically-detailed mathematical models, describing the ionic dynamics through algebraic and differential equations.

Continuous iterations between modelling, simulations and experimental work have progressively refined cardiac models over several decades [9–11]. As an example, in Fig. 1 (middle panel, top) is a cartoon representing the structure of the ToR-ORd model, a recently published computational model of human ventricular electrophysiology [12], based on the previous model by O'Hara et al. [13]. The ToR-ORd model was calibrated and evaluated using strictly separated processes and independent experimental datasets from human preparations, from ionic currents to whole-organ dynamics and the electrocardiogram. Specific aspects considered in its evaluation included drug-induced effects on the AP and the intracellular calcium, and the representation of diseased conditions, e.g. hyperkalaemia and hypertrophic cardiomyopathy.

Many types of compounds (e.g. antiarrhythmic drugs, antibiotics, cancer drugs, etc.) can affect cardiac electrophysiology, by interacting with one or several ion channels, and modifying their currents. This can be captured by a simple pore-block drug model [14], which computes

the residual current after drug application based on two experimentally-measured parameters: the half-maximum blockage concentration (IC_{50}) and the slope/Hill coefficient (h), according to the sigmoidal formula shown in Fig. 1 (left panel, bottom). More complicated approaches, e.g. Markov drug models, are also reported in literature [15,16]. The level of complexity required for computational modelling of drug action is still an open challenge, due to the high variability in drug response, as well as domains of applicability and research questions. Furthermore, patients can be prescribed more than one drug, especially in presence of multiple disorders, thus adding another layer of complexity due to possible drug-drug interactions.

2.2. A key year: 2013

In the last decade, an important novelty in cardiac modelling and simulation has been the focus on the investigation of sources and modulators of variability, starting from initial studies using sensitivity analysis [17,18]. For many years, experimental variability was considered a problem and a mere source of noise and uncertainty, to be removed in favour of mean values, small standard deviations and good p-values. However, the relevance of biological variability for disease and drug studies, and the importance to unravel its determinants became progressively established [9,19–22], in line with the needs identified in safety pharmacology [23] and for personalised medicine [24].

In 2013, we proposed a novel methodology to investigate the implication of biological variability in drug response [25], building on the work by Marder et al. in neuroscience [26]. This study was our first

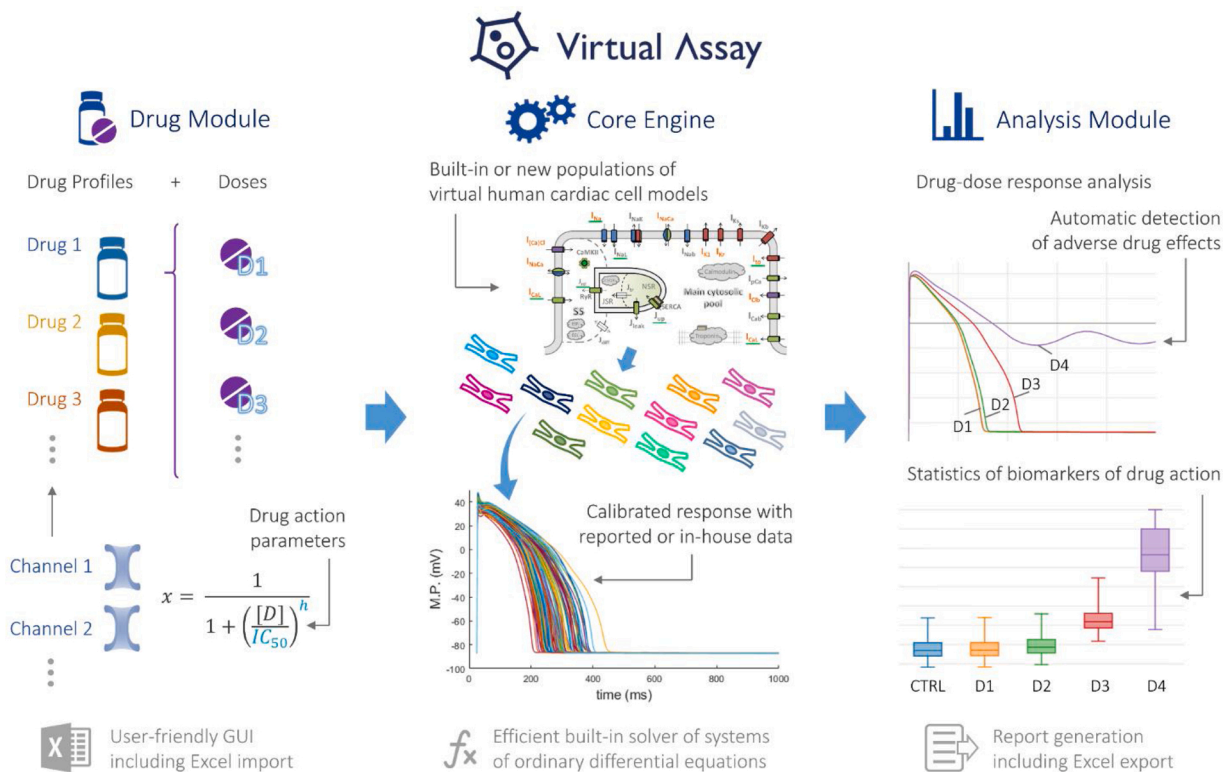


Fig. 1. Software design and operability in Virtual Assay. The *Core Engine* provides a user-friendly GUI to efficient algorithms for the sampling and solution of populations of virtual human cardiac cell models. Each model in the population (cells in different colours) is characterised by a different set of ion channel parameters (top inset), with biology described in the form of systems of ordinary differential equations, producing non identical action potential outputs to account for variability. Users can select between calibrating the physiological envelope of the models against their own in-house data, or use built-in populations. The *Drug Module* allows a user-friendly input of drug action parameters for an arbitrary number of drugs, including automated import from Excel files. The availability of each ion channel under drug action (x) is described by their respective IC_{50} and Hill (h) coefficients, as common in drug development practise. Each drug can be simulated at multiple doses $[D]$. The *Drug Module* directly converts the drug action parameters for their use by the *Core Engine* in each of the models of the population. The *Analysis Module* finally generates visual reports of the conducted drug-dose response studies, per individual model as well as providing statistics of biomarkers of drug action across the entire population, including the automatic detection of adverse drug effects. Reports can be exported in different formats for their subsequent analysis with other software tools. M.P.: Membrane Potential; CTRL: Control (no drug).

collaboration with Janssen Pharmaceutica NV (Belgium), who provided experimental AP recordings for the investigations. The methodology was termed experimentally-calibrated populations of models, and it consists of two steps: i) an initial population of models is generated, by randomly varying the model parameters; ii) a final population is obtained, by selecting only the models in agreement with the experimental data. An example of population of human ventricular models is shown in Fig. 1 (middle panel, bottom), where each coloured trace represents one cell in the population. All cells produce a similar AP, but the variability in the model parameters is leading to differences in shape/duration.

Due to its potential for the replacement of animal testing, the study by Britton et al. [25] received the 2014 NC3Rs (National Centre for the Replacement, Refinement and Reduction of Animals in Research) Prize.

Populations of models and variations of this approach have been applied to a variety of investigations using human atrial [27,28], ventricular [29–33], cardiac Purkinje [34], sino-atrial [35] cell models, human induced pluripotent stem cell-derived cardiomyocyte models [31,36,37] and also animal cell models [38].

Also in 2013 (July), the Comprehensive *in Vitro* Proarrhythmia Assay (CiPA) initiative was launched, following a workshop at the USA Food and Drug Administration (FDA) [39]. CiPA proposed a new paradigm to build on the maturity of computational human cardiac models to improve the accuracy of preclinical assessment of drug-induced pro-arrhythmia. In short, simulations of drug effects on the AP would be conducted based on experimental data of drug block on several ion channels as input. This aimed to overcome some of the limitations of the established paradigm, mostly focused around drug induced blockage of the hERG potassium channel and QT prolongation as proxy for pro-arrhythmic risk. Indeed, drugs that block hERG are not necessarily pro-arrhythmic, since many compounds have multi-channel actions, which can counteract the hERG blockage effects [39]. It was estimated that 60 % of new potential therapeutic molecules were abandoned early in development due to hERG concerns [40], in spite of not being necessarily risky. CiPA triggered interest both in academia and industry in the development and evaluation of computational approaches to safety pharmacology, using a variety of methods - see for instance [41–46].

2.3. Research innovation

With our collaborations in industry and the launch of CiPA, it became clear that our published methodology [25] had potential for uptake beyond academia. Therefore, we started our translation journey to transform our academic programming files into Virtual Assay, a user-friendly software tool for human *in silico* drug trials.

The structure of the Virtual Assay software is summarised in Fig. 1. The *Core Engine* (middle panel) consists of the ‘population of models’ methodology, already described above. The software includes by default human control populations of different sizes and cellular properties, all calibrated with human experimental data, but it also allows to create new ones based on reported or in-house data. A *Drug Module* (left panel) allows to simulate the effects on the populations of any tested drug, at multiple doses, using the simple pore-block drug model described above. Input data for this module are – for each drug – the testing doses and the experimentally-measured parameters that describe the effect of the drug on the cardiac ion channels of interest (IC₅₀ and h). The *Analysis Module* (right panel) elaborates and visualises the results of the drug simulations, comparing the different tested doses, and it also automatically detects and reports adverse drug effects.

For non-experts in modelling and simulation, Virtual Assay can be seen and used as a black box. For any tested drug, the inputs consist of information on drug/ionic current interactions, which are routinely measured in the early stages of the drug development process. The outputs are multiple figures and boxplots, showing the changes observed following drug application compared to control conditions, for a series of well-established cardiac biomarkers. For more expert users, Virtual

Assay offers the possibility of personalising the drug trials, by creating new populations of models, e.g. based on custom dataset or to represent specific diseases/mutations which could enhance/reduce drug effects, and also to save all the raw simulation data, which could be used for more detailed and complex investigations, as needed.

Virtual Assay has been developed in C++ and it uses the ordinary differential equation solver CVODE, part of the open-source Sundials suite [47]. Drug simulations in a modern laptop require approximately 5–10 min for each drug concentration for a population of 100 cell models, and simulations are run in parallel on multiple cores.

Two aspects facilitate the sustainability of the software: i) The Core Engine has been developed separately from the GUI, and this guarantees flexibility – we could create a new GUI for a different OS or online platform, for example; ii) New models and biomarkers – which are the key elements of our methodology - can be incorporated into the software in a modular way – without the need to change anything else. This guarantees that the software is always up to date, and it includes the latest published models.

3. Translation process

The translation journey that led to the development of Virtual Assay is illustrated in Fig. 2. With support from a first EPSRC (Engineering and Physical Sciences Research Council) Impact Acceleration Award grant, we had the research time and funding needed to develop the first version of Virtual Assay, which was released in 2014. At this stage, we were collaborating with three main industry partners. These early collaborations were crucial as they defined requirements through a questionnaire, provided valuable feedback on the software, with a better understanding of the industry perspective and of the current challenges in drug safety testing. Through a usability survey, we identified minor issues in the software, and we collected a series of suggestions on how to potentially improve its functionalities, e.g. by adding new biomarkers, new data analysis graphs, or an automatic import/export of data for bulk simulations.

We were successful in securing a second EPSRC Impact Acceleration Award grant to test the accuracy of the software predictions and refine the Virtual Assay software, with a new version released in 2016. We extended our network of collaborators by engaging with pharma companies and regulators. We presented the results of the evaluation studies performed using our methodology at industry-oriented and targeted conferences, e.g. the Safety Pharmacology Society Annual Meeting, the Gordon Research Conference in Drug Safety, and the regular meetings held by the FDA within the CiPA initiative. After establishing the initial collaborations, an increasing number of companies became interested in testing and evaluating the software.

At the same time, the award of a 5-year NC3Rs Infrastructure for Impact Award, provided the continuous support needed to accelerate the uptake of human-based *in silico* multiscale mechanistic methodologies for the evaluation of cardiac drug safety and efficacy in industry, regulatory and clinical settings. This allowed us to devote more resources to industry collaborations and validation studies, to continue to build confidence in our methodology.

In 2017, we published a key paper [48], demonstrating that human *in silico* drug trials using the Virtual Assay software reached close to 90 % accuracy in prediction of drug-induced pro-arrhythmic cardiotoxicity. The accuracy was higher than the one reported in studies using isolated rabbit hearts (overall accuracy of 75 %) [49,50]. In the same study, we also compared our results against two sets of experimental data, i.e. electrocardiogram in rabbit, and calcium recordings in human-induced pluripotent stem cell-derived cardiomyocytes, to highlight similarities and differences of *in silico* methodologies versus techniques more established in safety pharmacology. The study received the 2017 international NC3Rs prize and was also well received by the community, triggering additional studies with several pharma industry partners, published in the following years [51–58]. Free academic licences and

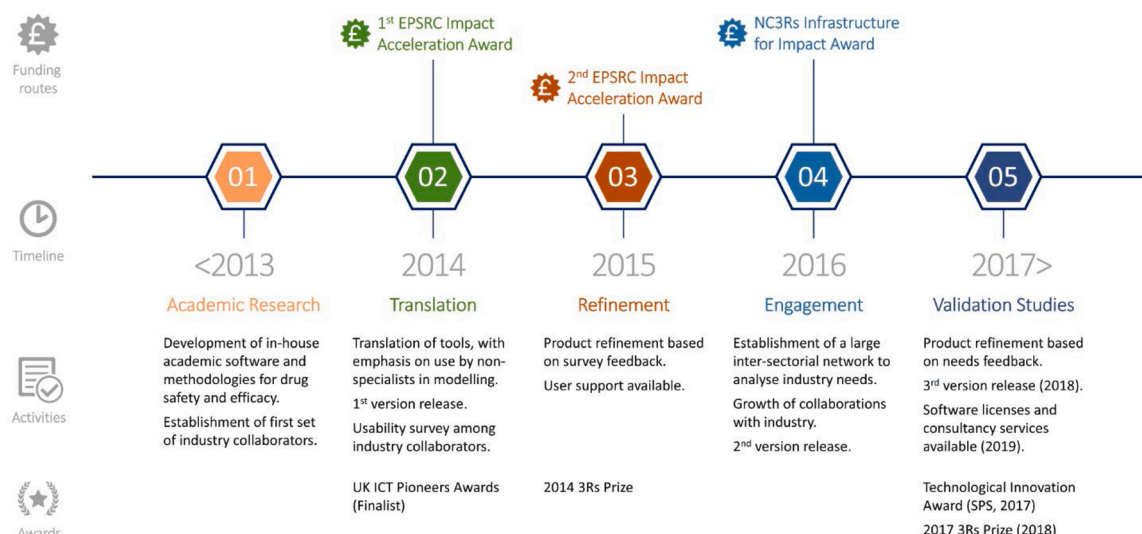


Fig. 2. Timeline of translational research process. Summary of awarded funding routes for translational research, phases in the translational research process, main activities per phase, and received recognitions. EPSRC: Engineering and Physical Sciences Research Council; NC3Rs: National Centre for the Replacement, Refinement, and Reduction (3Rs) of Animals in Research; SPS: Safety Pharmacology Society.

commercial licences are available through Oxford University Innovation (OUI) since 2019.

4. Impact and lessons learned

Fig. 3 summarises the domain of application and strengths of the Virtual Assay software, and its potential impact for drug evaluation. The key advantages are its user friendly interface and its ability to simulate drug effects on populations of human cells with incorporation of genetic diversity and disease (Fig. 3, top panel). This is indeed one of the limitations of animal models, which sometimes fail to detect potential adverse events in humans, even though they are still largely used for drug testing. Current strategies for drug cardiotoxicity assessment involve the selection of leading compounds through a combination of preclinical *in vitro*, *ex vivo*, and *in vivo* electrophysiological assays, using

a variety of animal species (rats, mice, rabbits, guinea-pigs). These experiments are performed at early stages of drug discovery, where many compounds are tested, and therefore this screening phase alone can easily exceed the use of 80,000 animals/year. Additional animal experiments, e.g. contractility assays in rats and *in vivo* QT assay in larger species such as dogs, are also performed, substantially increasing the number of animals used for drug safety studies (Fig. 3, bottom-left panel). The use of Virtual Assay could substantially reduce the number of animal experiments performed for the selection of leading compounds, and potentially completely replace *in vitro* and *ex vivo* animal studies for specific endpoints. Some of the companies using Virtual Assay are already implementing changes that could reduce their use for predictions of pro-arrhythmic cardiotoxicity. The integration of *in silico* methodologies in the drug assessment pipelines would improve the safety profile of candidate compounds in the pre-clinical phase of drug

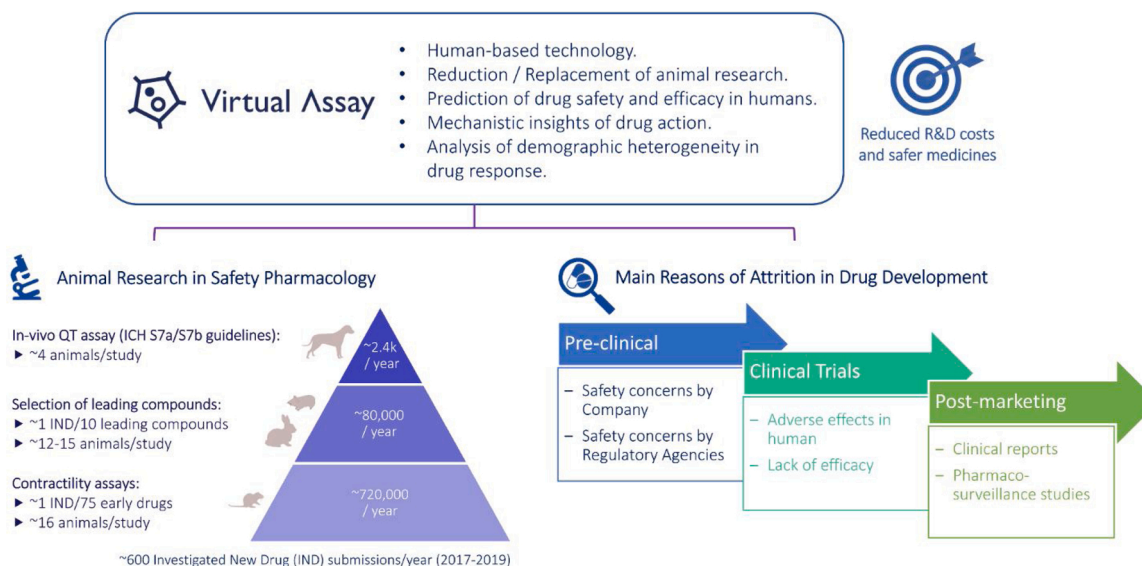


Fig. 3. Main domains of application of Virtual Assay. Virtual Assay aims to reduce research and development costs and to yield safer medicines in the drug development process. This is envisioned by the use of a mature human-based technology, allowing for the prediction of drug safety and efficacy directly in humans and therefore yielding to a net reduction and eventual replacement of animal research, and by providing mechanistic insights of drug action and demographic heterogeneity in drug response. 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).

development, thus reducing the risk of discovering new cardiac adverse events later on, during clinical trials in humans (Fig. 3, bottom-right panel). Ultimately, this will lead to safer medicines for patients, with an overall reduction in research and development costs.

In addition to the impact of our software in industry, our translation experience has also had an impact on our own research, and on the career development of our team members. Through discussions with our industrial collaborations, we became more aware of the current challenges and needs of pharma companies, and we had the opportunity to showcase our broader research on computational cardiovascular science, beyond human *in silico* drug trials. This extended our engagement with industry, leading to the development of new research questions and funding applications for future collaboration projects.

4.1. Challenges

The development of Virtual Assay required to overcome multiple challenges. First of all, simplicity was paramount to facilitate its uptake by non-experts in multiscale modelling and simulation. The human cardiac models embedded are biophysically-detailed, which allows mechanistic investigations, and considering populations of models provides advantages as well as added complexity. Therefore, a very simple GUI with clear options was a key requirement to fulfil. On the other hand, we wanted to make sure that our industry partners could understand the complexity of our research, beyond the Virtual Assay software, and we therefore engaged in further research collaborations with them.

A second challenge has been to make simulations quick and available in standard desktops or laptops. Therefore, we did multiple evaluation studies to try and optimise our drug trials, with the aim to reduce simulation time. We also chose to develop the GUI for Microsoft Windows OS, which seemed to be the one most commonly used based on the industry requirement survey.

A third challenge has been to provide continuity to the project over the years. The software translation was started by a PhD student (O. Britton), based on his first paper on population of models [25]. After working on the translation after his PhD, and contributing to the first version of the software, he was awarded a research grant to apply this methodology to pain research. The translation was then taken over by a postdoctoral scientist (E. Passini), who is still following the development of the software today. Other postdocs (X. Zhou and C. Trovato) joined along the way, contributing to evaluation studies, dissemination and software testing, and more people might become involved in future. B. Rodríguez and A. Bueno-Orovio supervised the project throughout, providing the stability and continuity needed to establish long lasting collaborations and kick-off the software uptake in pharma industry. For both students and postdocs, this experience contributed to a better understanding of industry needs, and of the differences between industry and academia. It also provided the opportunity to establish important collaborations, which influenced their future research directions and career development.

Finally, the biggest challenge has been in raising the credibility of human-based simulations as a useful tool, to complement the techniques currently used in preclinical drug development. While it was straightforward to prove that using computer modelling and simulations is cheaper and faster than animal experiments, we had to perform a thorough software verification, as well as validation studies, comparing our results both against the clinical evidence in humans and the results obtained in animal experiments. The validation studies considered different perspectives, using multiple types of animal/human data as well as different study designs, to suit the individual needs of our collaborators.

4.2. Lessons Learned

- It is important to have funding specifically designed to support translation, in order to be able to invest and dedicate time to the process beyond academic research. Allocated funding within a research grant or fellowship would facilitate the translation process in a more cohesive way. However, it is crucial to have the possibility of applying for external translation funding when this is not the case.
- Dissemination of the methodology and its evaluation studies, through journal publications and international targeted conferences, is crucial to enable institutional culture change – especially initially, when the methodology is still novel and not widely known nor in use in industry.
- Collaborations are essential to achieve impact, e.g. in our cases we shared our software with pharma companies, and we received valuable feedback as well as the experimental data necessary for validation.
- Simple is more: it is better to make sure the methodology/software is accessible and easy to use also by people with limited expertise in the field, while at the same time suggesting that more complicated methods are available if needed
- A clear record of all changes and updates facilitates the hand over when a software is developed by different people, as is the case with students and postdocs that can move on after a few years.
- Have the right expectations: a new method should not be judged by its own accuracy in isolation, but always in comparison with what is currently available.

5. Conclusions

We have described here our translational experience from the development of a research methodology to its uptake and impact beyond academia. The journey is far from linear. It has enabled rich inter-sectorial interactions, which have continuously fed back into our research, identifying and sharpening novel questions and ways of thinking. It has also enriched all parties' overall experience, and open up new job opportunities. In summary, it has had as much impact in our academic work as outside it.

CRedit authorship contribution statement

Elisa Passini: Software, Validation, Conceptualization, Writing - original draft, Writing - review & editing. **Xin Zhou:** Software, Validation, Writing - original draft, Writing - review & editing. **Cristian Trovato:** Software, Validation, Writing - original draft, Writing - review & editing. **Oliver J Britton:** Software, Validation, Conceptualization, Writing - original draft, Writing - review & editing. **Alfonso Bueno-Orovio:** Software, Validation, Conceptualization, Writing - original draft, Writing - review & editing. **Blanca Rodriguez:** Software, Validation, Conceptualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

EP, OJB, ABO and BR have worked with Oxford University Innovation (OUI) to develop and make available the Virtual assay software. The software is available via OUI for both commercial and academic research. XZ and CT declare no conflicts of interest.

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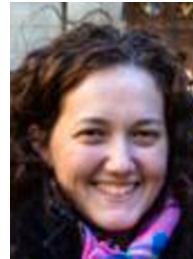
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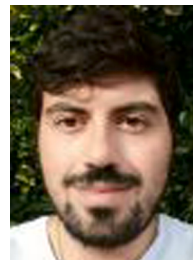
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