Avicenna Alliance
Association for Predictive Medicine

Avicenna Alliance Position Paper

The potential of *in silico* approaches to streamline drug development

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1. Contributions

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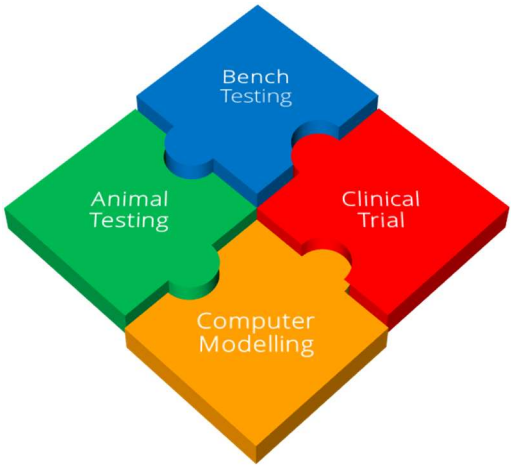
2. About the Avicenna Alliance

The Avicenna Alliance, is a non-profit global association of industry and renowned academia/healthcare organisations that have a commercial or research interest in the development of *in silico* medicine. Established in 2015, the Alliance has its origins in the Virtual Physiological Human Initiative, a European Commission (EC) endorsed research area on Computer Modelling & Simulation (CM&S).

This Avicenna Alliance bridges the gap between the scientific community, industry and policymakers by advocating for policy changes that take into account scientific and market developments. Its mission is to significantly accelerate medical innovation and its practical implementation, to ensure safe, affordable and cost-effective healthcare through the large-scale adoption of *in silico* medicine (CM&S).

The pharmaceutical community has been an early adopter of CM&S. The expression "*in silico*" methods was created in the pharmaceutical industry in 1989[1]. Today, up to 90% of the *in silico* models used in the pharmaceutical industry are statistics-based, molecular or data-driven models extracting equations from vast amounts of data and observations to drive drug discovery and development. In recent years, a growing fraction of physics- and physiology-based models have been used to complete existing approaches, especially for drug delivery and manufacturing. The *in silico* approach has been claimed by modellers and regulators to be a potential accelerator for the regulatory approval process and a strategy to substantially reduce cost and its environmental footprint of drug development process[2].



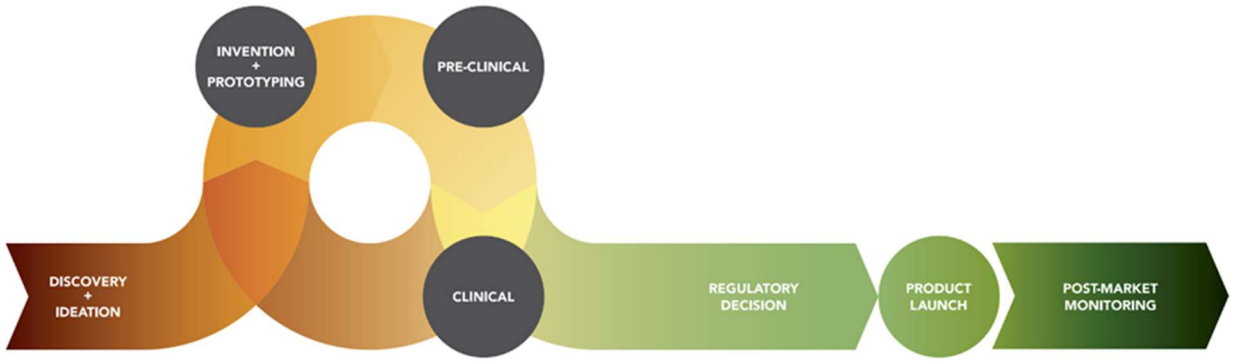
Computational modelling has the ability to support product development as a stand-alone form of evidence or in conjunction with already accepted forms of evidence.

[1] "DNA and RNA Physicochemical Constraints, Cellular Automata and Molecular Evolution", Pedro Miramontes, UNAM, "Cellular Automata: Theory and Applications" Workshop Los Alamos, New Mexico

[2] How Simulation Can Transform Regulatory Pathways | FDA

All sources of traditional evidence are useful models, i.e. abstractions of the reality of a complex situation helping to better understand the risk and benefits of a new solution. Bench testing and animal testing are obviously remote models of the target patients but nevertheless provide valuable information to understand a phenomenon or mechanism of interest. Clinical trials performed on relatively small populations are also models of the entire target population providing comprehensive information for the selected population. Similarly, widely validated computer models bring important insights of the drug, possibly interacting with a wide diversity of (virtual) patients, from the very early stages of the product development and regulatory approval processes. Only the combination of all these models provides the most comprehensive risk / benefit understanding of a new treatment.

While *in silico* methods are being used in the ideation, research and development, prototyping and testing of the medical solution development process in the pharmaceutical industry for decades, the impact of *in silico* method during the regulatory approval process or the consideration of digital evidence for regulatory decision, post-market surveillance or the investigation of adverse events still remain limited. The lack of local regulation for validating computational models and simulation and reporting digital evidence, not mentioning the absence of harmonisation between these different regulations have largely limited the usage of *in silico* approaches in the regulatory approval process for pharmacological products.



The Total Product Life Cycle of a medical product (reference: FDA. *Regulatory Science in FDA's Center for Devices and Radiological Health: A Vital Framework for Protecting and Promoting Public Health*. 2011 17 Sep 2018[3])

Considering the significant progress that has been made in advanced modelling methods and access to computational power, it is now possible to envision a future where drug programs will be maximised and clinical trials refined, augmented and eventually reduced by *in silico* clinical trials. More broadly, *in vitro*, animal and clinical testing could all benefit from computer models. At the recommendation of the EC, the Avicenna Alliance was created to consolidate the experience and expertise from the pharmaceutical, medical device and software communities, and suggest avenues to combine statistical and data driven approaches together with physics-based models. Some initial goals include accelerating the pace towards predictive and

[3] https://www.fda.gov/media/81709/download

personalised medicine for the patients as well as fostering medical innovation through comprehensive *in silico* trials, with the final goal of reducing the time, cost and environmental footprint of the regulatory approval process while increasing patient safety.

The mission of the Avicenna Alliance is articulated along 4 working groups:

1. The **Research and Technology working group**, whose mission is to bridge the gap between academic and industrial research and ensure that the technologies necessary to model complex physiological behaviours, are available or investigated by our large research communities from both academia and industry.
2. The **Policy Development working group** continuously interacts with European regulatory authorities and policy-makers to inform them about the potential and limitations of *in silico* methods, the *in silico* community about the priorities of the European authorities and streamline the discussion among them, the academic world and the industrial actors.
3. The **International working group** extends the collaboration with regulatory authorities to the rest of the world including USA, Asia-Pacific, and other global authorities. The goal of this activity is to converge towards a common framework that could eventually harmonise the different regulations.
4. The ***In Silico* Application working group** focuses on the concrete application of *in silico* methods in the day-to-day activities by leveraging the output of the three other working groups to continuously accelerate medical innovation for the benefit of patients.

3. Preface

3.1 Position paper objectives:

The COVID pandemic revealed clear weaknesses in the traditional approach to develop and test new drugs or vaccines, which cost $2 to 2.5 Bn over 10- to 15-year development cycles. Recognizing that this is not sustainable, a technology jump is necessary to maintain and increase the level of healthcare for future generations. *In silico* methods have been systematically used in various industries (including aeronautics, automotive, energy, high-tech) to accelerate innovation and have been used for over four decades by the pharmaceutical industry. In particular, *in silico* methods have been crucial to accelerate and amplify pharmaceutical innovation while increasing patient safety by increasing the number of *in silico* tests.

Despite increasing knowledge, the integration of digital evidence to streamline the regulatory approval process has yet to gain significant traction as of 2023. Synthetic control arms derived from real-world evidence have been used to support approval from both the FDA and the EMA for clinical study design for novel therapies; however, obtaining regulatory approval for new drugs or vaccines still entails extensive animal testing and clinical trials, which incurs a

significant cost and, more importantly, delays the availability of potentially life-saving treatments. Moreover, while digital evidence has been available, there is no legal framework to define how the existing models should be validated, how the digital evidence should be reported and how and by whom these data will be reviewed. For example, physics and physiology-based computational methods are not universally accepted yet, in part due to the fact that their validity relies on current scientific knowledge of existing processes. Knowledge-driven models could also be nicely complemented by data-driven models coming from observations and Artificial Intelligence (AI). As these methods are being developed, defining the context of use and demonstrating their validity for these specific questions of interest will be critical. All things considered, the absence of a clear process therefore prevents the large-scale adoption of *in silico* results for regulatory purposes, with the exception of PBPK modelling. Some initial efforts have already been made by the Alliance to build CM&S credibility in drug development[4]

Physics and physiology-based computational methods are not yet universally accepted, in part due to the fact that their validity relies on current scientific knowledge of existing processes. Although this knowledge is continuously increasing, knowledge-driven models could be nicely complemented by data-driven models coming from observations and Artificial Intelligence (AI). It is therefore extremely important to extensively define the context of use of *in silico* methods and demonstrate their validity.

All steps of the product life-cycle could widely benefit from *in silico* methods, including drug discovery, drug development, drug delivery and drug manufacturing. For example, CM&S would greatly benefit the processes of identifying the best delivery process for a given patient and testing the combination of drug and delivery device on representative target populations[5,6]. Similarly, the COVID pandemic has highlighted the critical need of rapid scale-up or adjustment of the drug manufacturing process to ensure rapid, and possibly local delivery of the treatment for the entire target population. Many efforts are being mobilised worldwide to invest and research on health technology consisting of *in silico* approaches[7].

[4]Musuamba FT, Skottheim Rusten I, Lesage R, Russo G, Bursi R, Emili L, Wangorsch G, Manolis E, Karlsson KE, Kulesza A, Courcelles E, Boissel JP, Rousseau CF, Voisin EM, Alessandrello R, Curado N, Dall'ara E, Rodriguez B, Pappalardo F, Geris L. Scientific and regulatory evaluation of mechanistic *in silico* drug and disease models in drug development: Building model credibility. CPT Pharmacometrics Syst Pharmacol. 2021 Aug;10(8):804-825. doi: 10.1002/psp4.12669. Epub 2021 Jul 13. PMID: 34102034; PMCID: PMC8376137.

[5] Lindsay E. Clegg, Feilim Mac Gabhann, Molecular mechanism matters: Benefits of mechanistic computational models for drug development,Pharmacological Research,Volume 99,2015,Pages 149-154,ISSN 1043-6618, https://doi.org/10.1016/j.phrs.2015.06.002.

[6] T.I. Adelusi, A.Q.K. Oyedele, I.D. Boyenle, A.T. Ogunlana, R.O. Adeyemi, C.D. Ukachi, M.O. Idris, O.T. Olaoba, I.O. Adedotun, O.E. Kolawole, Y. Xiaoxing, M. Abdul-Hammed,Molecular modeling in drug discovery, Inform. Med. Unlocked, 29 (2022) 100880–100818

[7] Leo CG, Tumolo MR, Sabina S, Colella R, Recchia V, Ponzini G, Fotiadis DI, Bodini A, Mincarone P. Health Technology Assessment for *In Silico* Medicine: Social, Ethical and Legal Aspects. Int J Environ Res Public Health. 2022 Jan 28;19(3):1510. doi: 10.3390/ijerph19031510. PMID: 35162529; PMCID: PMC8835251.

One of the projects funded by the EC, EDITH, is aimed at establishing infrastructure for digital technology for the healthcare and developing a virtual human twin[8].

3.2 Target audience:

There are numerous stakeholders engaged in the consideration of *in silico* methods for regulatory purposes, starting with the pharmaceutical companies, researchers, regulatory authorities, and ethics committees. Payers, patients and medical staff also represent important stakeholders.

- Regulatory Authorities

European regulatory authorities are fully aware of the potential brought by CM&S; they are keen to fulfil their mission to maximise patient safety while stimulating and facilitating safe medical innovation. Indeed, many regulatory authorities are actively engaging and progressing towards a careful adoption of *in silico* methods, as illustrated by the EMA's Regulatory Science Strategy to 2025[9] or the recently implemented Model-Informed Drug Development (MIDD) in the US[10]. Reporting, synthesising progress, illustrating successful case studies are relevant activities to provide them the necessary information for progressing in this important journey.

- Patients

Patients are central to any healthcare related issue as patients would eventually pay the price of delayed innovation or unsafe treatments. Alternatively, they can benefit from the impact of newer, safer, and more affordable healthcare. It is therefore essential to inform patient organisations about the potential and limitations of *in silico* methods, carefully listen to their concerns and involve them in communication and decision processes.

- Ethics Committees

Ethics committees have a dual role to play in this healthcare evolution. On one hand, it is crucial that new technologies, such as CM&S, are thoroughly investigated in terms of risks, benefits, and limitations for the patients to ensure a valuable outcome. On the other hand, *in silico* methods are the most promising approach to minimise animal testing and reduce the testing of unapproved treatments with real patients.

[8] https://www.edith-csa.eu/edith/

[9] https://www.ema.europa.eu/en/documents/report/emas-regulatory-science-strategy-2025-mid-point-achievements-end-2022_en.pdf

[10] https://www.fda.gov/drugs/development-resources/model-informed-drug-development-paired-meeting-program

- Health Technology Assessment (HTA) bodies /Payers

In silico methods provide much more insights about the interaction between new drugs and the body, which opens the way to more personalised diagnosis and treatment. These can potentially assist with dose adjustment as well as reduce the development cost and the risk of failure after large investment.

- Clinicians / Medical staff

The perspective of personalising treatments and reducing the risk of administering unapproved treatment to patients is in line with the expectations of clinicians and medical staff. Numerous clinicians are welcoming *in silico* methods as an additional tool to provide useful and validated insights to guide decision-making process.

- Industry

The pharmaceutical industry widely uses *in silico* methods during drug discovery and development, identifying and optimising drug delivery platforms, and the scale-up and optimisation of drug manufacturing processes. The opportunity to use digital evidence coming from fully validated computer models is expected to reduce production costs, minimise the risk of failure and dramatically cut time to market.

- Academics

For several decades, academics have been developing and improving advanced models to better understand the interaction of new drugs with the body and quantify their impact on various pathologies. The careful acceptance of extensively validated models will shed more light on their fundamental work and reveal and prioritise their most critical limitations.

3.3 Overview of position paper content:

This paper intends to reach out to all the stakeholders in the drug development industry to understand and encourage the adaptation of *in silico* technologies. It attempts to shed light on the evolution and application of CM&S so far in the drug development industry and look into the future possibilities and perspectives. It demonstrates the advantages of CM&S combined with traditional methods in optimising the drug development process. Recent developments in the drug development environment are looked into, in order to evaluate applicability and utility of CM&S. It also addresses the various kinds of models, modelling techniques and tools used for informing drug development process

The paper also encapsulates the gaps and limitations encountered in the environment that prevents the full extent capitalization of *in silico* technology to accelerate the drug development process and to mitigate the difficulties encountered. Finally, the paper emphasises the socio-economic and technological importance of the *in silico* methods

application in the drug development industry and calls for increasing the adoption of CM&S by developing industrial infrastructure, facilitating collaboration and implementing regulatory frameworks.

4. Introduction

Many successful attempts have been made to globally streamline the research, drug regulatory approval and quality requirements for clinical trials. Nevertheless, timelines and costs associated with drug development are constantly increasing. Over 90% of drug candidates which enter clinical trials fail in Phase 2 or Phase 3 due to adverse events or lack of efficacy[11,12,13]. This could be partially explained by the relative inaccuracy of animal models to fully represent human diseases especially in the case of drug development involving application of novel biologics (eg, cell and gene therapies). Of course, the use of human tissue *in vitro* is relevant, but it poses many technical issues (tissue source, survival, etc.). *In silico*, in combination with *in vitro*, *ex vivo* and *in vivo* models, can strengthen the bridge between nonclinical and clinical contexts and provide a more complete understanding of drug product efficacy and safety[14] .

For ethical, societal, regulatory and financial reasons, CM&S strategies should be applied more broadly as it has the potential to help overcome the current socio-economic and technological barriers to medical innovation. For example, CM&S can be used as an exploratory tool to guide the design and execution of studies during drug development and to decrease the burden on patients and the costs associated with drug development.

The development of a new drug relies on an integrated approach involving Chemistry, Manufacturing, and Control (CMC), nonclinical (NC) and clinical studies. During product development, *in silico* approaches can help in addressing many challenges. One example is the use of CM&S to evaluate and adjust in-process controls during manufacturing. *In silico* approaches such as AI-driven or hybrid-modelling-driven avenues could be a viable option to accelerate CMC development alongside Ways-of-Working (WoW) through the Quality by Design (QbD) framework. Several NC challenges can also be addressed using *in silico* approaches (e.g. early pharmacology, pharmacokinetics (PK), safety). Although CM&S is not widely applied in all these areas of drug development regulatory approval, some tools and techniques are already well established and accepted by regulatory agencies, such as population pharmacokinetics

[11] Dowden H, Munro J. Trends in clinical success rates and therapeutic focus. Nat Rev Drug Discov. 2019 Jul;18(7):495-496. doi: 10.1038/d41573-019-00074-z. PMID: 31267067.

[12] Arrowsmith J, Miller P. Trial watch: phase II and phase III attrition rates 2011-2012. Nat Rev Drug Discov. 2013 Aug;12(8):569. doi: 10.1038/nrd4090. PMID: 23903212.

[13] Hay M, Thomas DW, Craighead JL, Economides C, Rosenthal J. Clinical development success rates for investigational drugs. Nat Biotechnol. 2014 Jan;32(1):40-51. doi: 10.1038/nbt.2786. PMID: 24406927.

[14]Zhou Z, Zhu J, Jiang M, Sang L, Hao K, He H. The Combination of Cell Cultured Technology and *In Silico* Model to Inform the Drug Development. Pharmaceutics. 2021 May 12;13(5):704. doi: 10.3390/pharmaceutics13050704. PMID: 34065907; PMCID: PMC8151315.

(popPK, by EMA, FDA), PBPK (by EMA, FDA) or Quantitative Structure-Activity Relationship (QSAR by FDA)[15]. Nevertheless, guidance for the use of emergent technologies (i.e. CM&S, *in silico* trials and Artificial Intelligence (AI)/Machine Learning (ML)) to support medicinal product development is still missing. For the moment, sponsors tend to limit *in silico* approaches to CMC and early NC studies. Because of the uncertainty of the acceptance of digital evidence by the competent authorities, only a few sponsors submit digital evidence as the main supportive data[16].

The classic approach in drug development has led to the need for large animal numbers. To mitigate this, the international community implemented various legislations and guidelines based on the 3Rs principle (Replacement, Reduction, Refinement), such as the Directive 2010/63/EU and ICH M3 guideline[17]. Animal use will unfortunately remain necessary during the development of a new drug, especially for testing safety, but CM&S approaches will help to replace and refine some of the animal testing and de-risk nonclinical studies by optimising the choice of the species, refine study designs or even include animal virtual populations. We anticipate that the impact and importance of *in silico* approaches on the nonclinical drug assessment will gradually increase. In time, the right balance between required nonclinical data and the possibility to apply *in silico* approaches must be determined for each drug product. *In silico* approaches will also leverage human clinical data already available (real-world data) that will complement the NC data. Real-world evidence is currently underused in the discovery-to-market process of a new drug. To build a model, all possible fit-for-purpose data should be used.

Development of a new drug requires documentation of its efficacy and safety in the target patient population. This evidence is typically collected through placebo-controlled, randomised, double-blind clinical trials with a statistically determined sample size. This step is a *sine qua non* condition to obtain regulatory approval and marketing authorization and reimbursement. Clinical trials are performed according to the globally accepted quality standard of “Good Clinical Practice” and to the principle of “what matters to patients” to best protect the participating patients while generating data supporting the treatment suitability for other patients. However, information on new treatments efficacy and safety is limited to the size and characteristics of patient populations and of the conditions studied in pre-authorization clinical trials. New indications, different patient populations and/or new formulations will often require additional clinical trials and thus additional economical efforts, limiting in many cases full exploitation of a drug's potential. In addition, the challenging issue of surrogate endpoints impairs the feasibility of trials since clinical outcomes require longer follow-up and larger samples. The correlation approach, the only one enabled by the current clinical research methodology cannot address this issue. Although new regulations have addressed the need to globally streamline regulatory and quality requirements for clinical trials, the time required and the costs associated with clinical development are constantly increasing. In addition, with new therapeutic approaches coming closer to personalised medicine, the current methodology of clinical research becomes less suitable. The

[15] Ekins S, Mestres J, Testa B. *In silico* pharmacology for drug discovery: methods for virtual ligand screening and profiling. Br J Pharmacol. 2007 Sep;152(1):9-20. doi: 10.1038/sj.bjp.0707305. Epub 2007 Jun 4. PMID: 17549047; PMCID: PMC1978274.

[16] https://www.fda.gov/science-research/about-science-research-fda/modeling-simulation-fda

[17] https://database.ich.org/sites/default/files/M3_R2__Guideline.pdf

low output of the medicinal product development decision-making process has been shown to result from low predictability of the current tools[18]. As a result, the number of new molecular entities per billion US dollars spent in R&D has been constantly decreasing since the 1950s, crossing the limit of one new drug approved per billion dollars spent in the late '90s[19]. More recent studies suggest that this negative trend continues unaltered[20]. The rising costs of clinical R&D present a barrier to innovation[21], with the risk that drugs with higher potential for return on investment will attract investments, independently of societal and patient needs.

Beyond their impact on R&D costs, clinical trials present several ethical issues that call for a paradigm shift:

- Patients, especially paediatric and elderly populations, often wait an unacceptably long time to have access to new treatment options.
- Patients with rare diseases are a particularly fragile population, not of economical interest for pharmaceutical companies.
- Target populations are not precisely enough defined, diluting the population of responders and possibly biassing conclusions regarding treatment efficacy22.
- Clinical trials are typically carried out on a population of Caucasian patients, disregarding the different responses that may exist between ethnicities due to biological differences (expression and activity of certain enzymes, morphophysiology, etc.). Indeed, recent studies have shown that the representation of Asian, African, Pacific islanders, Hispanic patients and others remain low compared to Caucasian patients, despite improvements in ethnicity/race reporting23,24.

CM&S has the potential to overcome these ethical, societal, regulatory and financial barriers if applied more broadly and used in the initial development phases to guide the design and execution of clinical trials.

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[18] Scannell JW, Bosley J. When Quality Beats Quantity: Decision Theory, Drug Discovery, and the Reproducibility Crisis. PLoS One. 2016 Feb 10;11(2):e0147215. doi: 10.1371/journal.pone.0147215. PMID: 26863229; PMCID: PMC4749240.

[19] Boston Consulting Group (2011). "Life Sciences R&D: Changing the Innovation Equation in India Delivering Affordable Innovation Through Global Partnerships". WebContent. https://www.bcg.com/documents/file80247.pdf

[20] DiMasi JA, Grabowski HG, Hansen, RW (2016). Innovation in the pharmaceutical industry: New estimates of R&D costs. Journal of Health Economics, 47:20-33.

[21] Scannell JW, Blanckley A, Boldon H, Warrington B (2012). Diagnosing the decline in pharmaceutical R&D efficiency. Nature Reviews Drug Discovery, 11: 191–200.

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[23] Ma MA, Gutiérrez DE, Frausto JM, Al-Delaimy WK. Minority Representation in Clinical Trials in the United States: Trends Over the Past 25 Years. Mayo Clin Proc. 2021 Jan;96(1):264-266. doi: 10.1016/j.mayocp.2020.10.027. PMID: 33413830.

[24] Loree JM, Anand S, Dasari A, Unger JM, Gothwal A, Ellis LM, Varadhachary G, Kopetz S, Overman MJ, Raghav K. Disparity of Race Reporting and Representation in Clinical Trials Leading to Cancer Drug Approvals From 2008 to 2018. JAMA Oncol. 2019 Oct 1;5(10):e191870. doi: 10.1001/jamaoncol.2019.1870. Epub 2019 Oct 10. PMID: 31415071; PMCID: PMC6696743.

5. Current use of *in silico* evidence in drug development and registration

The pharmaceutical industry ranks highest in Research and Development (R&D) consumption globally with close to 17% of worldwide revenues invested in R&D, followed by the tech industry (GAFA) with 8%, and aerospace with 6%; and yet pharma is a relatively late adopter to utilise CM&S at the heart of its overall drug development process. Pharma R&D involves merging of some of the most advanced *in silico* medicine technologies from the 1990s[25] with clinical testing practices that are over 2000 years old[26]. The need for greater adoption of CM&S techniques was a strategic component in FDA's Critical Path Initiative in 2004[27] and the more recent EU Innovative Medicines Initiative, especially the Drug Disease Model Resources (DDMoRe). It was amongst others seen as an instrument that could help address productivity declines at pharmaceutical companies as well as the ensuing decrease in regulatory approvals around the turn of the century. This began with the seminal paper of Sheiner (1997), where he described the overall drug development process as a series of 'learn' and 'confirm' cycles, where the emphasis during the 'learn' stage is on model-based methods aimed at integrating knowledge across studies, and drug development stages to maximise the insights gained from experiments. This integration is one of the true benefits of CM&S, though it comes at the cost of being clear about the (many) assumptions required to build these models[8]. If set up in a clever way, by accounting for the existing knowledge about the drug and the disease, these mathematical models (mechanism-based models) can help characterise emerging knowledge and new data and provide insights about the underlying physiological (body) system, which enables predictions for unstudied 'new' situations thereby informing decision-making. These mechanism-based models can be classified as Knowledge-Based (KBM) or Data-Driven (DDM), or a combination of these approaches such as population PK-PD models. The predictions will have to be confirmed by generating new data, which ultimately may also point to the need for further adaptations of the models developed (learn-confirm cycle). The advantage is that these assumption-rich models can help separate random, unexplained 'noise' (variability) from signal, and point to factors (often called covariates in DDM) that may explain part of this noise and hence be used to inform decision-making. The use of these types of mechanistic models also allow pooling information across trials, doses, populations and even across compounds with the similar mechanism of action, which can help inform decisions with more certainty or design experiments to decrease uncertainty about highly influential parameters in the model. Nowadays, Quantitative Systems Pharmacology (QSP) models have taken this knowledge driven approach to a certain extent, assuming that parts of the disease system are known from available knowledge (KBM). QSP can enable forward and backward integration of information across R&D programs with similar

[25] Insigneo.(2018). "*In Silico* Medicine: Definition, History, Institutions, Main Achievements". WebContent

[26] Avicenna (980-1037). "A treatise on the Canon of Medicine of Avicenna". WebContent. http://data.nur.nu/Kutub/English/Avicen- na_Canon-of-Medicine_text.pdf

[27] FDA (2004) Innovation or Stagnation? Challenge and Opportunity on the Critical Path to New Medical Products.

mechanisms of action (including back-ups) and rationalise target, compound and patient selection. This leads to knowledge management and modelling platforms such as Jinko®. Another example of such a CM&S approach is Physiologically-Based Pharmacokinetics (PBPK), which can predict compound PKs before they are studied *in vivo* based on the compound's physico-chemical characteristics and limited *in vitro* experimental data. It again starts from knowledge that has been built often over decades on the body and its physiological systems and whose parameters are fixed in softwares like Simcyp®, PK-Sim® or GastroPlus®. PBPK is currently used to predict DDIs, extrapolate PK to special populations (Japanese, paediatric, renal and hepatically impaired, etc.), inform formulation switches, etc. Beside the above stated examples, application of CM&S in the drug development cycle include other categories of modelling, like agent-based modelling, finite element modelling, logical modelling, etc. In the fields of autoimmune diseases, a typical example of agent-based modelling is UISS-MS, a tool for quantitative prediction of relapse rate and immune dynamics in virtual relapsing-remitting multiple sclerosis patients when exposed to specific treatments[28].

It is clear that CM&S spans the entire drug development cascade, from drug discovery, development, market access, commercialisation and life-cycle management, and via the simulation of different scenarios can address aspects such as dose/regimen selection, trial design, disease progression, placebo effects, special populations, formulation switches, positioning versus competition etc[29,30]. As a result, and because of the growing complexity and size of the data that are generated as part of the drug discovery and development process, CM&S, formerly also known as M&S (Modelling and Simulation), MBDD (Model Based Drug Development), MIDD and more recently MIDDD(Model Informed Drug Discovery and Development) is gaining traction[23]. CM&S is thought capable of informing decision-making at every step of a drug's R&D process including registration[21], both by the pharmaceutical industry, but also by global regulatory authorities, and is increasingly showing its value[31,21].

The benefits of the application of CM&S are increasingly being recognized. For example, Chabaud S (2002) informed the Phase 3 RCTs (Randomised Controlled Trials) using PBPK approach to predict the dose-effect relation of a new compound on the prevention of effort angina pectoris[32]. Dronne MA (2006), thanks to a model of acute ischemic stroke, showed why more than 300

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compounds clinical development failed[33]. Morgan et al. (2012) demonstrated a link between a higher probability of success in Phase 2 and having an integrated quantitative understanding of fundamental PKPD principles (Pfizer)[34]. Milligan et al. (2013) report a similar trend in late-stage clinical development productivity (successful Phase 3 and 4 trials at Pfizer)[35]. Visser et al. (2013) observed similar benefits for their Drug Discovery phase (AstraZeneca)[22]. These are only a few examples of the many that have been published meanwhile, where CM&S can sometimes be used to fill relevant data gaps as was the case for the SGLT2i inhibitor canagliflozin[36].

Regulatory Authorities have also been advocates of a broader application of M&S in Drug Discovery & Development, by putting forward Guidance documents on a range of topics of interest to Drug Industry, stressing the importance of a clear communication on assumptions, approaches applied and results obtained (population PK (FDA 1999, EMA 2007), exposure-response relationships (FDA 2003), PBPK reporting (FDA Aug 2018, EMA Dec 2018)). They have also published a number of summary articles on the use of M&S in Regulatory Decision making, covering the breadth of approaches available[37,38,39].

Specific regulatory guideline documents exist for methodological validation and reporting of Quantitative Structure-Activity Relationship (QSAR) methods as well as some pharmacometric approaches, such as population-pharmacokinetics (popPK), pharmacokinetics/pharmacodynamics (PK/PD), and dose/ exposure-response (DER) models. In contrast, regulatory guidance on knowledge driven models is scarce. The EMA[40] and FDA[41]

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[41] https://www.fda.gov/media/101469/download

physiology-based guidelines on pharmacokinetics (PBPK) models can be considered, up to now, as pioneers in this domain.

The M&S workshop organised concurrently by the European Medicines Agency (EMA) and the European Federation of Pharmaceutical Industries and Associations (EFPIA) in 2011 led to the installation of a M&S Working Group at EMA, and the publication of an EFPIA-initiated 'Good Practices document on MIDDD, which was subsequently endorsed by EMA[42]. However, those initiatives tended to overlook the whole compendium of *in silico* models while focusing on the most commonly accepted PK/PD and QSP models. The EMA working group has more recently been integrated into a broader Methodologies Working Party[43]. In addition, EMA offers a continuous tool to provide scientific advice and qualification opinion on novel methodologies for medicine development. Public releases of opinion letters indicate that qualified *in silico* model-based methodologies exist but remain scarce (e.g. MCP-Mod for phase II dose fitting studies, data driven model of disease progression and trial evaluation in mild and moderate Alzheimer's disease, Model-based Clinical Trial Simulation Platform (CTSP) for Duchenne Muscular Dystrophy, etc.[44]). Meanwhile, the Japanese Regulatory Authorities (PMDA) also put their supportive perspective forward[45].

Areas to further invest in are for instance, predicting the treatment effects in different patient populations and exploring comparisons with more concurrent treatments. This is possible today and is currently already recognized as an additional element of data generation in the drug development plan[46]. One of the examples of *in silico* exploration is taking PBPK to the next level of PBPKPD (Physiologically Based Pharmacokinetics and pharmacodynamics), using available knowledge on normal and pathological biology and physiology, which is a next step in the continuum[47]. Said this, this does not restrict the *in silico* approaches to extend its full potential applicable to all the *in silico* methodology spectrum not just PBPK based modelling. Besides, clarity on the circumstances upon which CM&S can replace actual clinical trials, what constitutes an acceptable and verified model, are next on the agenda of the Pharma/Regulatory interaction and have been taken up by ICH meanwhile.

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[43] https://www.ema.europa.eu/en/committees/working-parties-other-groups/chmp/methodology-working-party

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6. Challenges of new drug developments - Summary of identified gaps to enable *in silico* approaches in drug development

The increasingly highly diverse landscape of *in silico* approaches making their way from areas other than the "traditional" ones such as biophysics, bioengineering and biology into Drug Discovery Development and AI.

A pressing need for CM&S in orphan diseases and the paediatric/foetal patient population

Small patient populations present a real problem under current clinical development practices. Statistical analyses are restricted by small sample sizes, leading to non-significant trial results and to focus on non-clinical outcomes. Often, a control arm is not feasible nor ethically allowed. In addition, a small patient market translates in a reduced commercial interest for the pharmaceutical industry, leaving no therapeutic options to vulnerable populations, including patients suffering from orphan diseases and paediatric patients. For these patients, adding or replacing part of the clinical development with *in silico* clinical trial elements is not only a matter of financial benefit, it is also a matter of necessity.

Towards CM&S in orphan medicines development

The Orphan Medicinal Products Regulation was specifically designed to provide a market incentive to develop treatments for patients with rare diseases (i.e. low frequency) representing smaller markets (Regulation EC 141/2000). A revision of this legislation should maintain this spirit of incentivisation, by providing researchers with clarity about the regulators' requirements for acceptable CM&S application in support of efficacy and safety demonstration of their products.

Towards CM&S in paediatric/foetal medicines development

Clinical trials for paediatric and foetal patients are further limited by the need to comply with a higher patient safety threshold. Today, less than 10% of the total number of clinical trials are performed on paediatric populations (World Health Organization) despite nearly 30% of the world's population being children, and even fewer trials are performed in subgroups that most urgently need clinical innovations (e.g. younger children). Given the limited number of patients available, CM&S could support paediatric clinical trials by creating populations of virtual paediatric patients with set variables matching routinely and historically acquired clinical data, for the evaluation of a drug on a larger virtual population. While the advantages of this method are clear, work has to be done, like for rare diseases, to provide a regulatory framework to the use of *in silico* analysis when tackling paediatric diseases. *In silico* clinical trials in this scenario represent an ethical obligation given their potential to add a new digital layer of protection to children.

Towards CM&S in cancer therapy development

The complexity and heterogeneity typical of some particularly aggressive forms of malignant cancer, prevent traditional *in vitro* and *in vivo* approaches from focusing on more than one or two key features at a time, making it virtually impossible to get an overall view of this group of diseases. This may often result in the failure of the recommended treatments. This particular issue can be overcome through the application of CM&S strategies. Hence, numerous mathematical and computational approaches have been used in basic cancer research in recent decades. Being able to take into accounts several factors at once, along with all their interactions, *in silico* models can help researchers and clinicians in forming a complete picture of the disease and in making new hypothesis which can guide them towards a deeper comprehension of cancer[48].

Recently, the European funded project Primage[49] illustrated how CM&S could be used to accurately predict the growth of an abdominal cancerous tumour for a teenager. Comparison with clinical data shows a good qualitative agreement in terms of tumour volume growth. By incorporating the chemotherapy treatment in the model, the tumour shrinkage under the action of specific treatment was successfully modelled.

CM&S can assist in interpreting complex system structures and their underlying mechanisms, thus allowing the generation of valid preclinical and clinical evidence. CM&S applications range from biomarkers discovery and validation, making predictions about the effects of new/existing treatments and preclinical and clinical testing. This combination of clinical evidence and digital evidence opens the door to potential and promising digital twins of cancerous tumours.

Moreover, *in silico* approaches are fundamental to reduce laboratory work and, consequently, the number of animal experiments: the usage of a CM&S approach may help in the selection of the best experimental condition to be tested *in vivo*, thus saving money, time and animal distress[50], and effectively succeeding in the 3Rs (reduction, refinement, replacement) realisation[51].
For example, in the view to reduce the number of doses and optimise the efficacy of cancer preventive vaccinations[52], the use of *in silico* models allows to dramatically decrease the number of transgenic mice needed for a trial-and-error vaccination protocol.

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6.1 One example of orphan drug

Hypoparathyroidism (HypoPT) is a rare endocrine disease characterised by insufficient levels of circulating parathyroid hormone (PTH), leading to hypocalcemia and hyperphosphatemia. Hypoparathyroidism is a serious condition leading to severe deterioration of quality of life and can even be life-threatening.

Currently, the standard of care for hypoparathyroidism relies on correcting hypocalcemia with oral supplementation in calcium and vitamin D. However, this conventional treatment does not fully replace the functions of PTH and long term exposure to SOC in HypoPT patients not adequately controlled (NAC) with SOC may lead to episodes of hypercalcemia and hypercalciuria which combined with the disease-related hyperphosphatemia are all known risk factors for long term renal complications such as nephrocalcinosis, kidney stones, and renal deficiency.

A recombinant human parathyroid hormone (rhPTH(1-84)), acting as a substitutive hormone, has demonstrated significant reduction of SOC supplementation while improving control of calcemia in NAC patients[53] and real world research showed a favourable 5-yr impact on renal function. Evidence is still required to demonstrate long term impact of rhPTH(1-84) on renal function though long term double blind randomised clinical trials are not feasible in this rare condition.

An *in silico* study was designed to assess the occurrence of End-Stage Kidney Disease (ESKD) after 20yrs of treatment with rhPTH(1-84) compared to SOC in NAC patients. The study is a 20-yr follow-up, 3-arm *in silico* clinical trial, applying a calibrated and validated computational model4 built from knowledge about the pathophysiological mechanisms of HypoPT and chronic kidney disease to a population of virtual HypoPT patients (VP) NAC after 1-year of optimised SOC. Study compared 3 treatments, with each virtual patient receiving each of the 3, thus being his/her own control: SOC alone (control) for 20yrs (Arm1), rhPTH(1-84) alone for 20yrs supplemented with SOC when calcemia is uncontrolled (Arm2), SOC for 10yrs then rhPTH(1-84) as add-on to down titrated SOC for 10yrs (Arm3). rhPTH(1-84) and SOC were titrated to maintain calcemia within the target range 1.8-2.65 mmol/L. This study showed that in NAC patients, rhPTH(1-84) given as soon as SOC is confirmed inadequate, significantly lowered the incidence of ESKD and/or delayed its occurrence. Moreover, rhPTH(1-84) significantly lowered the mean eGFR decline whatever the history of SOC.

This long term study is the first *in silico* trial, based on an adequately validated computational model, conducted in HypoPT patients to complement short term clinical trials and support value demonstration of an orphan drug.

[53] Mannstadt M, Clarke BL, Vokes T, Brandi ML, Ranganath L, Fraser WD, Lakatos P, Bajnok L, Garceau R, Mosekilde L, Lagast H, Shoback D, Bilezikian JP. Efficacy and safety of recombinant human parathyroid hormone (1-84) in hypoparathyroidism (REPLACE): a double-blind, placebo-controlled, randomised, phase 3 study. Lancet Diabetes Endocrinol. 2013 Dec;1(4):275-83. doi: 10.1016/S2213-8587(13)70106-2. Epub 2013 Oct 7. Erratum in: Lancet Diabetes Endocrinol. 2014 Jan;2(1):e3. Dosage error in article text. PMID: 24622413.

6.2 One example of ATMP

Advanced Therapy Medicinal Products (ATMPs) are a class of innovative medicines recognized by regulatory agencies[54] that use biological products, instead of chemicals for traditional drugs, to treat diseases. Typical examples of ATMPS are gene therapies, cell therapies, or tissue engineering constructs. They are often considered as highly promising therapies to solve unmet medical needs and cure diseases (e.g tisagenlecleucel-T, Kymriah[55] or axicabtagene ciloleucel, Yescarta[56]).

They are characterised by their unique mechanisms of action, complex manufacturing processes, and high cost since the primary source of material comes from living tissues or organisms. Hence, some of those products can still "live and grow" in the patient's body. Due to their novelty, there are limited data available on their efficacy and safety, and they often face regulatory and commercialization challenges or the product is authorised for patients with poor outcomes while the safety must be monitored closely and continuously post-market approval[57]. Hence, the role that CM&S can play to address those challenges is even more critical than for traditional medicines.

Similar to other medicines, CM&S can be used to simulate the biological mechanisms of action of ATMPs and predict their efficacy and safety in silico. This can help to identify potential issues with the therapy and optimise treatment parameters before testing the ATMP in vivo or in clinical trials, in order to de-risk or reduce those experimental trials. In particular, modelling and simulation can help to identify optimal dosing regimens, patient selection criteria, and trial endpoints to reduce the risk of failed clinical trials or refine the scope of already approved medicines.

Currently, QSP approaches remain the main type of CM&S method used and accepted for that endeavour in a regulatory setting. However, in silico methods specifically qualified for that purpose are still missing. A typical context of use in which QSP modelling may be considered for ATMPs pertains to cell therapy safety evaluation. For example, chimeric antigen receptor T-cell (CART) therapy was envisioned to treat cancer patients because scientists anticipated a high efficacy, however, a life-threatening toxicity due to proinflammatory cytokine release syndrome (CRS) in CART-treated patients highlighted the possible danger of this new therapy. Hence some of those therapies are approved under a restricted program including a risk evaluation mitigation strategy[58]. A QSP model for the CART therapy has been developed to evaluate the inflammatory cytokine release in patients

[54] https://www.ema.europa.eu/en/human-regulatory/marketing-authorisation/advanced-therapies/advanced-therapy-classification
[55] tisagenlecleucel-T Kymriah; EMA info page: https://www.ema.europa.eu/en/medicines/human/EPAR/kymriah
[56]axicabtagene ciloleucel, Yescart; EMA info page: https://www.ema.europa.eu/en/medicines/human/EPAR/yescarta
[57] https://www.ema.europa.eu/en/medicines/human/EPAR/kymriah
[58] Yip A, Webster RM. The market for chimeric antigen receptor T cell therapies. Nat Rev Drug Discov. 2018 Mar;17(3):161-162. doi: 10.1038/nrd.2017.266. Epub 2018 Jan 29. PMID: 29375140.

with advanced chronic lymphocytic leukaemia (CLL) treated with anti-CD19 CART therapy59. The authors used data from a small clinical study to inform the development of the model, which described the complex relationships of CART and proinflammatory cytokines associated with CRS for CART therapy. It included parameters such as CAR-T cell expansion, cytokine secretion induction, and the pharmacokinetics and pharmacodynamics of the therapy. An external validation demonstrated they were able to predict clinical outcomes in terms of cytokine release based on patient-specific factors, such as disease burden baseline, and treatment parameters (e.g. dose). Similar types of models have also been considered to evaluate the efficacy of ATMPs, this is the case of another QSP model that was used to simulate patient responses to CART cell immunotherapy and predict the survival outcomes of patients based on different treatment scenarios60.

On a different note, the manufacturing process of ATMPs can also be optimised with advanced CM&S methods, potentially reducing the associated cost that often limit the scalability of ATMPs. For instance, for tissue engineering constructs, the scaffold shape (e.g. metallic or ceramic based) may be optimised computationally to allow for the most optimal tissue growth61. Although, to date and to the author's knowledge no computational models has been qualified by EMA as a method to develop tissue engineering construct (e.g. under the "Qualification of novel methodologies for medicine development" tool62).

6.3 One example of paediatric development

Atopic dermatitis (AD), also known as eczema and atopic eczema, is one of the most common inflammatory disorders, affecting up to 20% of children and 10% of adults in high income countries[63]. AD causes considerable morbidity, imposes a high economic burden[64] and cannot be cured at present[65]. The main hurdle in AD clinical development is the occurrence of patient

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[62]https://www.ema.europa.eu/en/human-regulatory/research-development/scientific-advice-protocol-assistance/qualification-novel-methodologies-medicine-development-0

[63]Langan SM, Irvine AD, Weidinger S. Atopic dermatitis. Lancet. 2020 Aug 1;396(10247):345-360. doi: 10.1016/S0140-6736(20)31286-1. Erratum in: Lancet. 2020 Sep 12;396(10253):758. PMID: 32738956.

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[65] Schmitt J, Schwarz K, Baurecht H, Hotze M, Fölster-Holst R, Rodríguez E, Lee YAE, Franke A, Degenhardt F, Lieb W, Gieger C, Kabesch M, Nöthen MM, Irvine AD, McLean WHI, Deckert S, Stephan V, Schwarz P, Aringer M, Novak N, Weidinger S. Atopic dermatitis is associated with an increased risk for rheumatoid arthritis and inflammatory bowel

heterogeneity due the prevalence of multiple phenotypes[66]. To ensure efficient administration of given treatment, profiling the best responders is very helpful. The techniques involved in identifying patient traits include invasive methods like tissue biopsy which is very hard to be performed in paediatric populations. Thus, an *in silico* exploration tool is developed to meet the needs of optimising clinical development.

In silico platform for paediatric atopic dermatitis can simulate a wide spectrum of AD phenotype[67]. The model design focuses on young children with low to moderate AD severity and can be used to predict disease severity and skin biomarkers. Simulation of investigational treatment, prediction of the best responding phenotype and identification of the leading biomarkers will lead to optimization of treatment development for AD paediatric population. The *in silico* platform can also be extrapolated to the adult population for testing the Investigational treatment for different known phenotypes that would increase chances of success of a clinical trial.

7. COVID example

Acute viral respiratory tract infections (RTIs) are the main cause for wheezing in preschool children and are associated with a high degree of acute inflammation. Recurrent RTIs lead to exacerbation of the damages and thus increase the risk of developing permanent wheeze or asthma due to airway remodelling. Efficient strategies to prevent or reduce frequency of viral RTIs in children at risk for recurrent infections is currently an unmet need[68]. However, trials investigating interventions against respiratory diseases were profoundly affected by non-pharmaceutical interventions (NPIs) against COVID-19 which perturbed existing regular patterns of all seasonal viral epidemics.

A modelling approach was designed to simulate RTI prophylaxis trials with paediatric patients under COVID-19 pandemic conditions to assess trial feasibility and better inform trial design and clinical development[69]. The developed knowledge driven model couples a within-host viral

disease, and a decreased risk for type 1 diabetes. J Allergy Clin Immunol. 2016 Jan;137(1):130-136. doi: 10.1016/j.jaci.2015.06.029. Epub 2015 Aug 4. PMID: 26253344.

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[68] Rossi GA, Pohunek P, Feleszko W, Ballarini S, Colin AA. Viral infections and wheezing-asthma inception in childhood: is there a role for immunomodulation by oral bacterial lysates? Clin Transl Allergy. 2020 Jun 3;10:17. doi: 10.1186/s13601-020-00322-1. PMID: 32509272; PMCID: PMC7255835.

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infection model with an epidemiological between-host model of RTIs transmission. The model was calibrated to reproduce the age-dependent seasonality of the main respiratory viruses with and without enforced NPIs. After matching intra- and inter-patient variability in RTI resolution and efficacy data, the model was used to simulate placebo-controlled *in silico* trials in 1 - 5-year old paediatric patients with recurrent RTIs (RRTI) treated with an immunomodulating bacterial lysate under 4 different hypotheses of NPI intensities and assessed efficacy and benefit metrics as a function of NPI intensity.

Model's predictions showed that sample size estimates based on the ratio of RTI rates (or the post-hoc power of fixed sample size trials) are not majorly impacted under NPIs which are less severe than a strict lockdown. However, NPIs show a stronger impact on metrics more relevant for assessing the clinical relevance of the effect such as absolute benefit. Furthermore, the simulations showed that a mild NPI scenario already affected the time to recruit significantly when sticking to eligibility criteria complying with historical data. In conclusion, the model allowed the design of COVID-19 pandemic related risk mitigation strategies for efficacy confirmation trials concerning this class of products and respiratory tract infection prophylaxis trials in children in general.

8. Challenges in implementation of *in silico* approaches

In silico approaches refer to computational techniques that are used to simulate and model biological systems. These approaches have many potential benefits, such as faster insight generation and cost-effectiveness over traditional experimental techniques, they come along with certain challenges. Some of the challenges are stated below:

- **Accuracy limited to the extent of reliability of data**: validity of *in silico* approaches are limited by the accuracy of the data and assumptions that are used to develop the models. If the models are not based on reliable data, they may lack complete ability to represent the biological system being studied and reproduce desired behaviour[70]. This possibility holds true to the animal models also where the considered animal model for preclinical studies may not be a complete replacement for the human subject. This challenge can be managed by proper verification and uncertainty quantification to validate the utility of the model.
- **Need for experimental validation**: *in silico* approaches are often used as a first step in the research process, but they still require experimental validation to confirm their accuracy. Without experimental validation, it can be difficult to determine if the models accurately

[70] Adikesavan, A. K., & Katkoori, S. (2020). *In Silico* modelling in cancer research: challenges and opportunities. Frontiers in Bioengineering and Biotechnology, 8, 943. doi: 10.3389/fbioe.2020.00943

reflect the behaviour of the biological system[71].One enabling step would be to multiply the number of validation datasets available to the community, such as the recently released HFValid collection [72]

- **Simplification of biological systems**: *in silico* approaches often simplify the complexity of biological systems in order to make them conceptually and computationally tractable. While it is easier to create simplified models, possibility of oversimplification and the loss of important details need to be paid attention[73]. This is why existing validation frameworks rely on the definition of a precise context of use for the model. Indeed the model can only be validated and used within a predefined context, which accounts for the inner model's assumptions and limitations. It is important to incorporate all the necessary elements of the biological system being modelled in the CM&S process.

- **Need for computational resources:** *in silico* approaches often require significant computational resources, raising a need for efficient ecological and economic management of the working environment. This can limit their applicability to research groups or institutions with compatible computing power and resources[74]. This highlights the need for democratising the access to large public European computational infrastructures.

- **Limited ability to account for all factors**: biological systems are complex and influenced by many factors, such as environmental conditions and genetic variability. *In silico* approaches may not be able to account for all of these factors, which can limit their applicability and in certain cases their accuracy[75]. Hence, various approaches have been proposed to carefully quantify uncertainties (systematic error and random error).

In summary, the *in silico* approaches are limited by the fact that they are an abstraction of biological systems, and the accuracy of the results is heavily dependent on the assumptions and data inputs used to construct the model. These limitations can be mitigated to a great extent by considering the *in silico* approach along with the traditional approach in a complementary manner. *In silico* technology should be used to overcome the challenges in traditional approaches harmonising the efforts and in an inter supportive manner to address the hurdles in drug development process as a whole ecosystem consisting of all the different approaches. As such, in a more conservative standpoint, it is recommended to use *in silico* approaches in conjunction

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[72] Aldieri, Alessandra ; La Mattina, Antonino Amedeo ; Szyszko, Julia Aleksandra ; Baruffaldi, Fabio ; Viceconti, Marco (2022) HFValid collection: Hip-Fracture validation collection. University of Bologna. DOI 10.6092/unibo/amsacta/7126.

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[74] Hjelm, R. D., Catanzaro, B., Cho, K., Jakovetić, D., Soh, H., & Bengio, Y. (2019). Federated learning for healthcare informatics. Journal of Medical Internet Research, 21(3), e12663. doi: 10.2196/12663

[75] Bawa-Khalfe, T., & Cheng, J. (2016). A study on the challenges of integrating big data in healthcare. Journal of Healthcare Engineering, 2016, 1-10. doi: 10.1155/2016/9061502

with validation and to carefully consider the challenges and potential sources of error inherent in these approaches.

Some of the known examples of challenges of *in silico* approaches in specific area of drug development are further discussed below:

In drug discovery: Drug discovery has suffered a myriad of changes in the last three decades in the way of adopting the prediction of a compound likelihood to be successful, or conversely enable identification of molecules with liabilities as early as possible. Among the changes, include the integration of *in silico* approaches for the design and optimisation as complementary roles to traditional *in vitro* and *in vivo* approaches[76].

There is a general understanding with regard to *in silico* approaches that they should be accompanied by further *in vitro* and *in vivo* experiments to verify the biological activities. Nevertheless, there are lots of identified compounds by *in silico* screening methods which do not have a correspondent *in vitro* or *in vivo* evaluations to prove the real positive response. For instance, *in silico* molecular approaches are utilised to make modelling for toxicity pathways, especially when there is a lack of essential experimental data available[77,78]. The lack of correspondence between *in silico* with the experimental methods, might be seen by those whose illiteracy justifies the bad judgement, as not trustworthy, damaging the credibility of the evidence based on *in silico* methods. For instance there is literature that exposes toxicology scientists thinking that it is not possible to replace animals thoroughly by *in vitro* and/or *in silico* studies in safety examination in the future[79].

In silico methods such as molecular docking offer a solution to drug development. For instance, CADD approaches and methods offer higher probabilities of identifying compounds with desired properties increasing the compound chances of overcoming the barriers of preclinical testing. But Ligand-based design (LB-CADD), finds its limitations of pharmacophore-LBDD (pharmacophore, i.e., the molecular unit responsible for specific biological interaction) by the complexity of the molecular dynamics. The computational demand and dependency of the size of simulated systems and analysis timing ranging from hundreds of nanoseconds to microseconds presents limitations, i.e., short times to analyse protein folding, resulting in inadequate sampling of protein conformations. One of the main limitations of molecular docking is ensuring appropriate scoring functions and algorithms (AI/ML) to be implemented, which may compromise molecular screening (based on several articles from Proventa International).

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[76] Sacan A, Ekins S, et al., applications and limitations of *In Silico* models in drug discovery, doi: 10.1007/978-1-61779-965_6

[77] McGovern SL, Shoichet BK. Information decay in molecular docking screens against holo, apo, and modeled conformations of enzymes. J Med Chem. 2003 Jul 3;46(14):2895-907. doi: 10.1021/jm0300330. PMID: 12825931.

[78] Robert J. Kavlock, Gerald Ankley, Jerry Blancato, Michael Breen, Rory Conolly, David Dix, Keith Houck, Elaine Hubal, Richard Judson, James Rabinowitz, Ann Richard, R. Woodrow Setzer, Imran Shah, Daniel Villeneuve, Eric Weber, Computational Toxicology—A State of the Science Mini Review, Toxicological Sciences, Volume 103, Issue 1, May 2008, Pages 14–27, https://doi.org/10.1093/toxsci/kfm297

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In general, as with any other approach, *in silico* based drug discovery and design encounter limitations on the quality of the data utilised in the computational method. High resolution data of target biomolecules is not always available, jeopardising the success of the molecule design process[80].

High resolution data and quality also is a limitation in next-generation sequencing ('NGS') modelling for clinical molecular diagnostic laboratories, in terms of limitations on the ability to detect variants or variety of types of *in silico* data against which aspects of the pipeline they can be validated. It is necessary to take into account that limitations can be found as well in the software to process the data. Furthermore, research on limitations showed that *in silico* generated NGS data files used to test pipelines performance encounter technical limitations, inadequate *in silico* data for a robust validation or do not represent essay correctly[81]. Some studies on NGS speak also on the limitations of the information contained in the rRNA sequence databases[82].

Virtual patients and synthetic data: With the application of the concept of virtual patients cohorts and the use of AI-based algorithms and synthetic data based on statistical properties of real world data, a limitation is found in the fact that "*while each synthetic data field will have the statistical properties of real data a the univariate level, the complex multivariable relationship between data fields will be difficult to capture. Thus, this approach would generally yield low- or medium-fidelity synthetic data*"[83].

Virology: In the virology field, one of the lessons from the COVID-19 pandemic for advancing computational drug repurposing comes from the host-targeting approaches which normally involve the integrations and analysis of multiple omics types and use data-driven network-based methods, which major limitation was found in the lack of gold-standard datasets and the scarcity of data from Middle East respiratory coronavirus and SARS-CoV outbreaks[84].

Altogether we can establish that a main common limitation *in silico* approaches is the lack of access to the information, and unavailability of proper standardised datasets.

9. Conclusion and recommendations

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[81] Eric J. Duncavage, Joshua F. Coleman, Monica E. de Baca, Sabah Kadri, Annette Leon, Mark Routbort, Somak Roy, Carlos J. Suarez, Chad Vanderbilt, Justin M. Zook, Recommendations for the Use of *in silico* Approaches for Next-Generation Sequencing Bioinformatic Pipeline Validation: A Joint Report of the Association for Molecular Pathology, Association for Pathology Informatics, and College of American Pathologists, The Journal of Molecular Diagnostics, Volume 25, Issue 1, 2023, Pages 3-16, ISSN 1525-1578, https://doi.org/10.1016/j.jmoldx.2022.09.007

[82] Regueira-Iglesias, A., Vázquez-González, L., Balsa-Castro, C. et al. *in silico* evaluation and selection of the best 16S rRNA gene primers for use in next-generation sequencing to detect oral bacteria and archaea. Microbiome 11, 58 (2023). https://doi.org/10.1186/s40168-023-01481-6

[83] Puja Myles et al 2023 Prog. Biomed. Eng. 5 013001

[84] Galindez, G., Matschinske, J., Rose, T.D. et al. Lessons from the COVID-19 pandemic for advancing computational drug repurposing strategies. Nat Comput Sci 1, 33–41 (2021). https://doi.org/10.1038/s43588-020-00007-6

In silico methods present an enormous potential to address some of the current challenges in drug development. Some avenues to promote adequate use of these methods include:

i) The implementation of an End-of-Phase 2A or MIDD meetings exclusively dedicated to *in silico* during the drug approval process at FDA and similar dedicated meetings at other regulatory agencies. Such meetings provide companies with a development pathway and a milestone platform where *in silico* strategies and activities for the dose finding, late development and registration processes of their compound to be discussed and agreed with authorities.

ii) The implementation of key binding text (*in silico* trial) in clinical development plans for agreed modelling and simulation activities with scope, trial requirements, validation criteria, timelines, etc.

ii) The addition of *in silico* expertise coming from scientific areas other than the "traditional" ones into the EMA Methodology Working Party or FDA Modeling & Simulation Working Group, enabling assessment of i*n silico* Development Plans and activities at large.

10. Definitions

Clinical outcome
Measurable change in patient's health, function or quality of life at specific time point.

Computational modelling and simulation (CM&S) in healthcare
Computational modelling and simulation (CM&S) makes use of programming languages, numerical methods, high-performance computing, to create numerical representations of first-principles equations, to reduce, refine or even replace experimental and clinical research.

Digital Evidence
Results of computational modelling and simulation submitted as part of a (regulatory) evaluation process provided in a prescribed format, that encompass *in silico* trials and *in silico* tests.

In silico
In silico means carried out in the computer (i.e., the silica), which is analogous to *in vitro* (in the glass), *ex vivo* (outside the living organism), or *in vivo* (inside the living organism).

***In silico* clinical trial (ISCT)**
Use of computational modelling and simulations to mimic the human experimentation required in the regulatory evaluation process of a medicinal (including medical devices) product or intervention, under well-defined conditions using verified and validated models. It requires the definition of virtual patients or virtual populations of patients.

(*Please refer to the glossary of term publication by Avicenna Alliance for more related definitions*[85]).

11. Resources

All the references are listed as footnotes.

12. Disclaimer

The views and opinions expressed in this position paper are those of the authors and do not necessarily reflect the views or positions of any entities they represent.

[85] https://avicenna-alliance.com/files/user_upload/Avicenna_Alliance_Glossary_of_Terms_for_Computer_Modeling_and_Simulation.pdf