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In-silico trials alternative *in-vivo* animal studies: potentiality, predictive modelling, and realism

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Abstract

The use of very powerful models can persuade the translation from labs and animal research as well as the human trials into something simpler, less tedious, and more precise, just as digitalization has tenfold transformed industries like financial services, insurance, entertainment, and tourism. In these times of pandemic, we have realized the value of new drug innovation. However, at the same moment, we all happened to know how the amount of time required for a particular medication to be developed. Our time is valuable, and we cannot afford to wait a few years for the establishment of a new medication that might fail. R&D spending is expected to cost approximately 400 to 500 crores. Up to 50% of the time and cost of medication and medical device production could be avoided using *In-silico* processes." The 3Rs, or refinement, reduction, and replacement reasoning represent the road to applying these strategies in a manner that guarantees appropriate outcomes that are as close to the real-world outcome as possible. Model validation is a crucial step in achieving this degree of consistency and offering the best solution to *In-vivo* animal experiments. This review article seeks to offer knowledge that can help clinical trials progress quicker and for less use of animals.

Keywords: *animal studies; clinical trials; in-silico trials; PBPK; QSAR; virtual modulation.*

1. Introduction

Healthcare education is faced with multiple global challenges difficulties (Kononowicz et al., 2019). Our capacity to glean empirical evidence collected from preclinical studies to human clinical procedures is poor due to a lack of systematic knowledge of animal models, which contributes to data misinterpretation and needless animal waste (Xing et al., 2016). It is important to implement a strategy that will speed up the rate of drug production while simultaneously protecting animals. There is a need for innovative methodologies that can reduce costs and increase the accuracy of clinical trials so that the healthcare sector can advance. The latest methods generating such hope do not consist of one-by-one substitutes of specific animal experiments but reflect a radically new, human-oriented, and systems-biology-centered approach to drug development, integrating a variety *of In-vitro*, *In-silico*, and human *In-vivo* methodologies. They're inspired by a need to cut costs and boost the pace and accuracy of drug production, as well as a deep desire for improvement (Archibald, Tsaioun, Kenna, & Pound, 2018). Although regulatory agencies allow drug and product developers to use modeling in their work, many do not have the appropriate IT infrastructure in place. *In-silico* Trials seeks to

resolve this concern by gathering the best modeling models from universities and embedding them in a modular IT cloud-based framework that can be used for several studies. Future potential for in vitro drug trials Human experiments *In-silico* for discovery and preclinical trials, the phenotype approach, and the target-based approach are the two key models used in the drug discovery phase, particularly in an industrial setting. The former is the one that has been pursued in the past. It involves searching for compounds that are graded based on their biochemical effects in several settings, ranging from cell-based phenotypic assays to isolated tissue or animal models of disease. As a result, future medications could show negative side effects. The target-based strategy, on the other hand, begins with proteins that have historically been connected to the human condition under investigation. The target could then be validated, and statistical simulation may be used to illustrate the relationship with the target in this situation (Pappalardo, Pennisi, Reche, & Russo, 2019; No authors listed, 2021). The so-called animal ban, which forbids animal experimentation for cosmetic purposes. So how can adverse effects of cosmetic products be predicted as the ban is for both marketing as well as testing? *In-silico* trials can be the answer for all these concerns by using computer modulation and predicting all the adverse events (Desprez et al., 2018).

In this pandemic scenario, COVID 19 is the most well-known disease currently, and *In-silico* trials can be a valuable method for drug growth, speeding clinical trials, and acting as a suitable alternative to *In-vivo* animal research (Srivastava et al., 2020). Simulation is just the first step toward prediction. In the absence of all available evidence, *In-silico* IVIVE is a simulation (Rostami-Hodjegan & Tucker, 2004). Figure 1 explains the design of *In-silico* and working of it containing simulation of protein, validation of the target, identification, simulation of PBPK, QSAR, 3D QSAR.

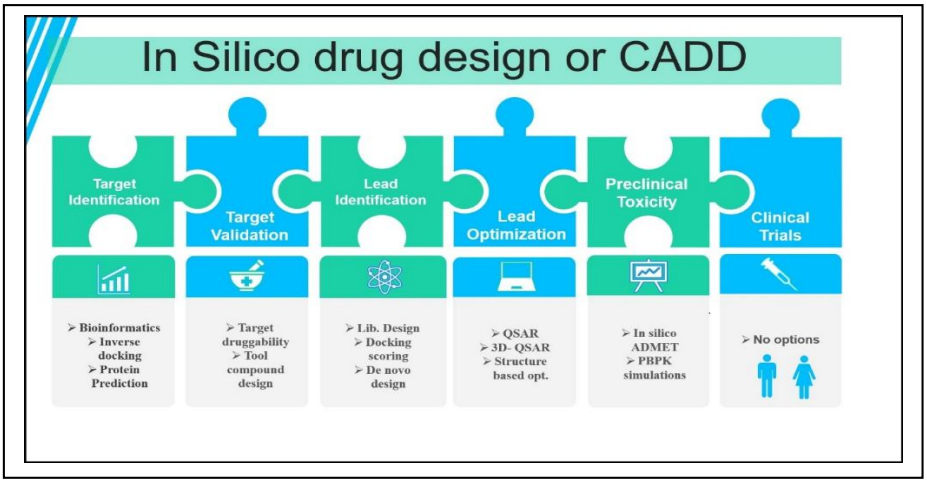

Figure 1 *In-silico* drug design

2. *In-silico* trials having virtual patients

In-silico trials are a cost-effective and legal way to test various care options, case studies (Mancini et al., 2018). Virtual patients, cohorts, and trials have the potential to advance better solutions by accounting for pathophysiological conditions as well as intra- and inter-patient variability in response to treatment. Because these latter changes in patient status cannot be predicted, they are difficult to control in a regular clinical trial. However, they can be easily found retrospectively in clinical evidence, and thereby *In-silico* in planning a clinical trial with a reasonably large simulated cohort. As a consequence, long before deployment, can guarantee that a procedure is accurate. As a consequence, a validated *In-silico* virtual research framework with suitable virtual cohorts will enable phase II/III human trials and preclinical trials to be removed or decreased in the amount (Chase et al., 2018). Digital populations are one method we've found to be useful in exploring mechanistic and parametric complexity in an intuitive context that most people can appreciate. Despite their extensive use, however, there are only a few documented methods for

building virtual patients and virtual communities in which *In-silico* trials are one of them (Morrison, Pathmanathan, Adwan, & Margerrison, 2018). *In-silico* clinical trials simulates the effects of a drug or biomedical system on real patients using computer models i.e., virtual patients. Physiologically dependent Pharmacokinetic/Pharmacodynamic (PBPK/PD) models, as well as software methods that help their development and automated analysis, are the pillars of *In-silico* clinical trials. The integration of modern computing technology with mathematical or theoretical characterizations of cancer cell biology, known as "*In-silico* experimentation," is an innovative approach to leading the early stages of theory creation and experimental design that reduces time, money in the lab, this computational approach is useful since it allows for the implementation of a huge range of experiments that can be observed at any degree of detail and replicated and controlled at will (Mallet, 2012; Kent, 2021).

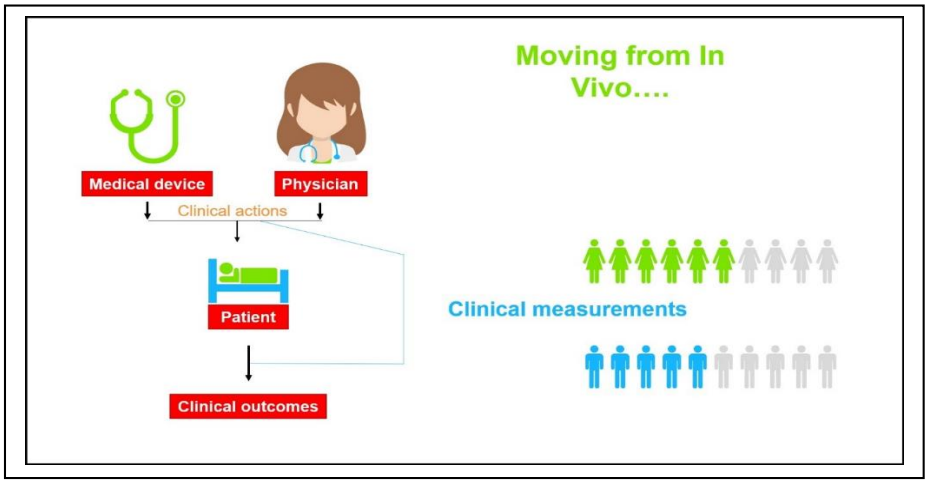

Figure 2 Progressing from *In-vivo*

The automatic numerical simulation of the device's actions in a large number of virtual patients, in which certain aspects of the outcome such as device protection or biomechanical effect are expected, and the subsequent statistical analysis of these theoretical effects, will constitute an i*n-silico* trial (Galbusera et al., 2018). Figure 2 and Figure 3 explain moving from *in-vivo* to i*n-silico* and how *in-silico* is beneficial than *in-vivo* studies.

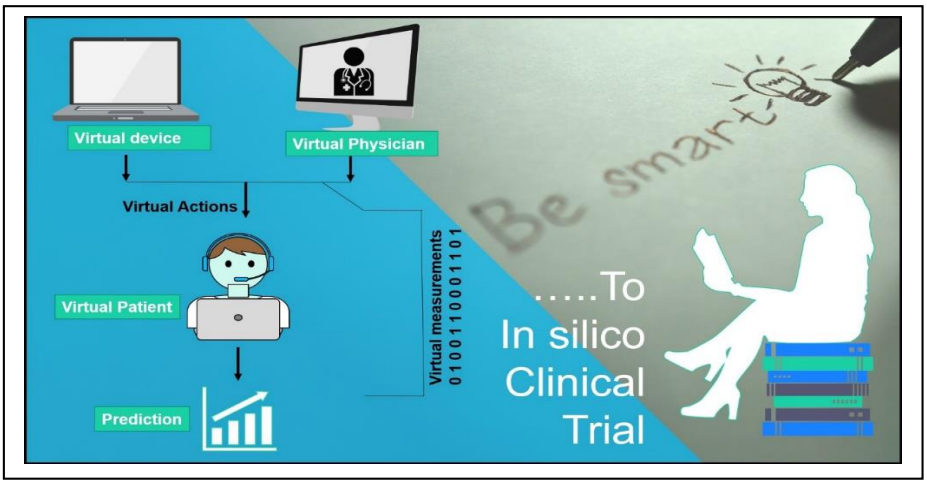

Figure 3 *In-silico* clinical trials

3. Chemical information databases

Scientific knowledge is being acquired at a faster rate than at any other time in history. Much of this new information must be made available in the public domain to maximize its value. This has resulted in the rapid growth of databases, many of which are open to the public. Interrogating past databases is a critical 1st step in analyzing a chemical's potential effects; if appropriate data is already available, testing or making predictions is unnecessary. In general, it is preferable to use an experimental method rather than a predicted method. If the information for the chemical of interest isn't available, it may be possible to predict by combining data from other chemicals; this procedure permeates *In-silico* modeling tools like QSAR and interprets (Madden, Enoch, Paini, & Cronin, 2020). COVID-19 drug discovery using chemical informatics can be done with help of QSAR (Hawłas & Lewenstain, 2011; Muñoz-Fontela et al., 2020; Naseri et al., 2020; Amin, Ghosh, Gayen, & Jha, 2020). Hence, to use *In-silico* trials chemical database use is very important.

4. Computer modulation and virtual screening

TGC protocols have been designed using virtual patient trials. The clinical outcomes of SPRINT demonstrated very near compliance with predicted simulation (Suhaimi et al., 2010; Alderisio, Lombardi, Fiore, & di Bernardo, 2017; Gomeni, Bani, D'Angeli, Corsi, & Bye, 2001). Digital patients, virtual reality role trainers, and serious gamers are also example of virtual worlds. While the terms "virtual space" and "virtual universe" are often used interchangeably when referring to virtual reality, there is a distinction to be made between the two. Virtual environments and realms are configurations for the virtual reality experience (Isaza-Restrepo, Gómez, Cifuentes, & Argüello, 2018; Pappalardo, Russo, Tshinanu, & Viceconti, 2019; Cant, Cooper, Sussex, & Bogossian, 2019). Modeling and simulation are powerful tools that the musculoskeletal biomechanics community has known and used (Erdemir, 2016). Experiments of serious conditions that are impractical in animals or clinically unlikely in humans may be conducted using simulation (Micheletto et al., 2013; Gumaste et al., 2020). PRIMAGE *In-silico* models to be developed require substantial computing and data storage resources to be processed which may be used to support childhood cancer (Geris, Lambrechts, Carlier, & Papantoniou, 2018; Viceconti et al., 2019; Martí-Bonmatí et al., 2020).

These techniques play important role in *In-silico* trials providing greater accuracy and could be the best alternative to *In-vivo* animal studies. Treatment results can also be estimated using statistical simulation.

5. *In-silico* and PBPK relation

The basic principle of the PBPK-concept is that a community of organs, a single organ, or a part of an organ is a chamber (compartment) with an entry and exit for blood as the primary material transport. The compound can remain in the compartment, pass through it, leave the organism, or participate in metabolism (Kormazeva & Soloviev, 2017). Several studies have shown that using clinical data, *In-silico* physiologically dependent pharmacokinetic (PBPK) modeling methods integrating ADME behavior can predict PK behavior in humans. A drug's physicochemical and physiological properties are used to build an *In-silico* PBPK model. It can provide a quantitative mechanistic paradigm for forecasting systemic and tissue exposures. If human PK activity can be reliably replicated as a basis for human bioequivalence (BE) studies using *In-silico* PBPK modeling techniques, a clinically applicable specification (CRS) can be developed based on simulation findings rather than clinical research results (Kato et al., 2020). In addition to education and training, the study is needed to make PBPK models more available and in user-friendly formats. Getting models in an easier-to-use, a simplified format capable of simulating real-world situations would significantly improve their utility and use, allowing creative techniques and technological advancements to be further incorporated into decision-making processes, PBPK can be used to measure the internal dosage of a mixture based on the interaction of the three chemicals in the mixture. Although drug activity can be modelled to some degree, vaticinating the effect of dose type on *In-vivo* results on a solely digital basis has proved difficult. In a 'predict, understand, and validate' paradigm, ''middle-out" methods are useful more recently. Subsequently, *In-vivo* data is useful to modify the current PBPK model, the ''middle-out" method seems to be the most realistic path forward in today's production paradigm (Kostewicz et al., 2014). PBPK models are a crucial component of drug growth. Without the use of PBPK models, *In-*

silico trials are seriously restricted. Knowing the ADME of the drug and how it will function in the body is important for predicting *In-vivo* trials. Knowing the pharmacokinetic drug activity prediction can be easily calculated. If it is used cautiously, *in-silico* determined specifications might become beneficial and permissible for PK estimation (Park et al., 2017). Models with minimal permeability in the kidneys examine the result of outward movement and assimilation carriers in the clutch of cells of concern (Martinez, Gehring, Mochel, Pade, & Pelligand, 2018). Hence, *In-silico* trials and PBPK are interlinked to each other. In a population of simulated "type 1 diabetic patients," the *In-silico* trial showed that linear performance input MPC achieves adequate glycemic control where the PBPK model played an important role (Magni et al., 2007). The sample size and heterogeneity (e.g., in anatomies) of the underlying patient cohort should be such that they represent real-life when planning *In-silico* trials. The sample size should be sufficient to identify significant effects of the extent expected in traditional clinical trials (Sarrami-Foroushani et al., 2021).

6. Quantitative structure-activity relationship (QSAR)

At its most fundamental form, it is a tool that is used for designing numerical that uses a chemometric methodology to try to discover by the use of statistics relevant association among configuration and functionality.

Almost all QSAR approaches aim to accomplish the following objectives:

- To clarify their properties related to chemicals they are more definitely determining factors for their physiological behaviors,
- To boost the biological processes of current leads by optimizing them,
- To anticipate the biological behaviors of molecules that have not yet been evaluated and are often unavailable (Verma, Khedkar, & Coutinho, 2010). Modern 3D QSAR methods calculate the interaction energy in a grid to analyze the interaction fields around a moiety.

This step is repeated with each of the molecules in the sequence, with the orientation of the molecules concerning one another being a key parameter. Each point in the grid's interaction energies is then subjected to a QSAR through the range of chemical moieties, QSAR enables medicinal chemists to scan a limitless "chemical and stereochemical space" for new "lead compounds" that can be used to produce potential drugs after a further study into ADME-Toxicological properties *viz.* adsorption, distribution, metabolism, excretion, and toxicity of drugs (Prajapat, Agarwal, & Talesara, 2017). The 4D-QSAR approach has proved to be both useful and accurate (Andrade, Pasqualoto, Ferreira, & Hopfinger, 2010). We could develop new drugs with higher activity and test them more quickly using QSAR models. Calculating methods have been used to investigate certain FDA-approved products, which have proven to have positive properties (Si, Xu, Hu, Si, & Zhai, 2021).

Table 1 QSAR Terms used

Terms	SI UNITS
Molar volume	(m^3/mol)
Boiling point	°C
Vapour pressure	(Pa)
Water solubility	ppm
Dissociation constants (pka)	Mol/L
Partition coefficient	No unit

Modeling QSAR with 3D Descriptors (3D QSAR). Many QSAR expressions mean that biological selectivity is the product of each target forming extremely complex associations with a ligand, such as hydrogen bonds and as a result, two new approaches for 3D QSAR alignments have emerged: the topomer protocol and the still-evolving "prototype" protocol. Both methods are incredibly simple, essentially transforming 3D QSAR from one of the most time-consuming and hence expensive CADD approaches into one of the simplest, on the verge of being almost fully automated (Knaak, Dary, Power, Thompson, & Blancato, 2004; Schmidt, Casey, Paterson, & Chan, 2013; Hamza, Salim, & Saeed, 2016; Jamei, 2016; Hayashi et al., 2019; Byeon et al., 2020).

7. Animal studies

Animal models are generally used in preclinical in vitro, ex vivo, and *In-vivo* science, but outcomes do not necessarily apply to humans (Cherkasov et al., 2014; Liu & Lv, 2018; Penha et al., 2020; Gumaste et al., 2020). In cruelty-free international Jarrod Bailey is a senior research scientist, He gave a lecture on “Non-human primates in neuroscience research”. According to the speaker, researchers often misinterpret the harm-gain analysis: the pain of the animals is underestimated, while the supposed benefit is

inflated. Deprivation of fluid and food, as well as head fixation, could result in a condition analogous to a human post-traumatic stress disorder, which may affect the outcomes. Human-centred in the other hand, imaging methods, neuroimaging, and cognitive science techniques are effective and applicable to humans (Doke & Dhawale, 2015; Carvalho et al., 2019; Passini et al., 2019). There was a workshop which was hosted by Lang et al., 2018) which featured sixteen attendees who were divided into 2 teams of professionals that were postdocs and Ph.D. candidates. The core task was posed to candidates which were "Design a plan to incorporate *In-silico* methods in basic science to support the 3Rs and the students asked a various question which revealed interesting aspects such as a) discomfort of researchers doing animal testing, b) a protective mentality when it comes to animal research, c) mistrust of animal welfare groups, d) a lack of awareness and faith *In-silico* approaches and alternatives in general, e) a lack of desire to speak about own experimental work and f) rage about the pressures of animal experiment implementations, including the defense against the 3Rs (Gericke & Strittmatter, 2019; Van Norman, 2020). Over the last quarter-century, the use of alternative approaches for product testing and preclinical testing of prescription products and equipment has grown significantly. The number of papers written using "alternative species" (e.g., mosquitoes, fish, worms, and shrimp) and *In-silico* research grew by over 900 percent between 1990 and 2015 and. *In-silico* modeling was used in over 88,000 experiments in 2015, compared to just 7,405 studies in 1990. During the same period, the use of guinea pigs and rabbits in cosmetic experimentation and study declined by 68 percent and 40 percent, respectively (Lang et al., 2018). Often animal models are too easy to reliably replicate human environments, in general, existing animal models do not reflect the sluggish, cumulative, and degenerative nature of many human chronic diseases, nor do they account for comorbidity or polypharmacy (human patients often take more than one type of medication) and failures of construct validity, which is commonly understood to be a subset of external validity, are often defined as failures of animal models to correctly reflect human diseases and clinical contexts (Pritzker, 1994; Ge et al., 2006).

Challenges and problems in animal studies:

a) Despite the high incidence of drug failure in clinical trials, preclinical testing is now regarded as the most important step in the drug development process.
b) Research issues
c) Societal and personal issues
d) Economic issues (Pound & Ritskes-Hoitinga, 2018; Fontana, Figueiredo, Martins, & Santos, 2020).

8. Application of *In-silico* trials

Perfusion for Acute Ischaemic Stroke *In-silico* Trials (Padmos, Józsa, El-Bouri, Payne, & Hoekstra, 2019) *In-silico* Trials used in vancomycin drug trials. *In-silico* design used for the Development of CK2 Inhibitors, Glycemic Control in Intensive Care: Generalizability of a Virtual Trials Method (Cozza, G. 2017; Dickson et al., 2017; Colin, Ponckheere, & Struys, 2018) *In-silico* Imaging Trial of Digital Breast Tomosynthesis as a Replacement for Full-Field Digital Mammography (Badano et al., 2018; Denkert et al., 2019). The use of particle therapy in the re-irradiation of patients with head and neck cancer has been demonstrated to be advantageous, according to the findings of a multicentric In-silico ROCOCO trial (Eekers et al., 2016). *In-silico* Trial of Hypofractionation in Advanced Non-Small-Cell Lung Cancer Radiotherapy (Hoffmann, Troost, Huizenga, Kaanders, & Bussink, 2012). pVAC-Seq; *In-silico* approach to detecting tumor neoantigens that are driven by the genome (Hundal et al., 2016). Antigenic Epitopes and The effects of drugs on bone remodeling were predicted *In-silico* instruments (Geris, 2020). SARS-CoV-2 RNA-based RNA polymerase targeting: *In-silico* perspective (Elfiky, 2020). *In-silico* analysis is used to compare mutants in cancer from drivers (Hanrahan et al., 2020). Determinants of combination GM-CSF immunotherapy and oncolytic virotherapy effectiveness found by personalization *In-silico* therapy (Cassidy & Craig, 2019). Alzheimer's disease: Amyloid beta-dependent pathogenesis and therapeutic effects, as well as *In-silico* interventions that emphasize the importance of natural products (Awasthi, Singh, Pandey, & Dwivedi, 2016). *In-silico* research on the anti-inflammatory properties of luteolin (Aziz, Kim, & Cho, 2018). Connecting Arterial Blood Flow to Tissue Design *In-silico* Analyses for a New Trial Recombinant Multiepitopic Rotaviral Vaccine (Jafarpour, Ayat, & Ahadi, 2015). Clinical Trial

Simulations in Children and the Glass Mouse Model: Identifying and Individualizing Optimal Isoniazid Doses in Tuberculosis Patients (Jeena, Bishai, Pasipanodya, & Gumbo, 2011). Implantable Cardioverter Defibrillator *In-silico* Pre-clinical Trials (Jiang et al., 2016). Official Siddha Formulation and JACOM were tested *In-silico* against the SARS-CoV-2 spike protein (Kiran et al., 2020) *Nigella sativa L* as a possible coronavirus disorder phytotherapy 2019 (Koshak & Koshak, 2020). Acute Vocal Fold Injury was used to test a patient-specific *In-silico* model of inflammation and healing (Li et al., 2008). Transcatheter aortic valve repair inspection using *In-silico* methods (Luraghi, Rodriguez Matas, & Migliavacca, 2021). An *In-silico* approach to evaluating the contentious results of clinical trials: a report on the complexities of temporary HIV treatment. PRIMAGE is a project that uses quantitative *In-silico* multiscale analytics to aid in the personalized diagnosis of children with cancer using imaging biomarkers. The Optimum Ratio for Pramlintide and Insulin Co-administration in Type 1 Diabetes was modeled in *In-silico* (Dassau et al., 2009; Haidar, Wilinska, Graveston, & Hovorka, 2013). Clinical assessment and real-time modeling of hepatic radiofrequency ablation using *In-silico* preparation [ClinicIMPPACT Trial] (Moche et al., 2020). All-Optical Electrophysiology refines *In-silico* Human iPSC-CM populations for drug testing (Paci et al., 2020) The Results of a Multicentric *In-silico* Clinical Trial Comparing Photon and Proton Radiotherapy for Non-small Cell Lung Cancer (Roelofs et al., 2012). Taking the next step in tuberculosis *In-silico* modeling: a collaboration with UISSTB (Russo et al. 2020). Acidic environment changes and Injection of food have no effect on the half-life of ribociclib: *In-silico* Studies (Samant et al., 2018). *In-silico* results towards a run-to-run adaptive artificial pancreas (Toffanin et al., 2017). Prescription of radiation doses for non–small-cell lung cancer based on natural tissue exposure constraints: *In-silico* experimentation (van Baardwijk et al., 2008). Models of acute inflammation in animals *In-silico* (Vodovotz et al., 2006). An *in-silico* research found that individualized isotoxic dosage prescribing improved the efficacy of stereotactic radiosurgery in massive brain metastases (Zindler, Schiffelers, Lambin, & Hoffmann, 2018). The viral evolutionary lineage is reconstructed simultaneously, resulting in a potent gene therapy vector (Zinn et al., 2015).

9. Conclusion

A debate on which method is superior, *In-vivo* animal experiments or *In-silico* trials, could go on for a long time with no clear conclusion. The only argument is that the world is shifting towards digitalization, and the health industry needs to consider the benefits of digitalization and how to use it to improve human life. While it is impossible to fully stop using animals in clinical trials, in many instances or situations, *In-silico* experiments have definitive outcomes at a much quicker pace. As previously said, about 50% of drug discovery projects fail, even though they are in phase 2 or phase 3 phases, and this is attributed to animal studies' inability to identify multiple adverse effects of the drug, or to put it another way, animal studies have struggled to predict the adverse effect of the drug on the human body in the vast majority of cases. *In-silico*, on the other hand, has shown its precision and can be very useful in speeding up clinical trials. The United States and the European Union have now begun to use *In-silico* trials as an alternative to *In-vivo* animal testing, and the expectation is that *In-silico* will become widely adopted, paving the way for a new age of clinical testing that is both effective and reliable. The association of statins with the key protease enzyme of SARS-CoV-2 was studied using *In-silico* molecular docking which turned out to be very useful in this pandemic situation (Reiner et al., 2020).

10. Future perspective

As in this pandemic era, we understood the need for successful drug development in a shorter period. *In-silico* trials provide a greater acceleration to the clinical trials which could lead to successful drug development in a short period. It's time for the health sector to implement new digitalization techniques for greater accuracy. *In-silico* trials are showing a very bright future and new hopes. Using these techniques, we could be ready for this type of pandemic situation in the future. Let us not be dependable only on animals for drug trials, humans need perfection and *In-silico* is providing that perfection.

11. Declaration of competing interest

The authors declare that they have no known competing financial interests or personal

relationships that could have appeared to influence the work reported in this paper.

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