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Review Article

Exploring the Power and Promise of In Silico Clinical Trials with Application in COVID-19 Infection

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Abstract

Background: COVID-19 pandemic has dramatically engulfed the world causing catastrophic damage to human society. Several therapeutic and vaccines have been suggested for the disease in the past months, with over 150 clinical trials currently running or under process. Nevertheless, these trials are extremely expensive and require a long time, which presents the need for alternative cost-effective methods to tackle this urgent requirement for validated therapeutics and vaccines. Bearing this in mind, here we assess the use of in silico clinical trials as a significant development in the field of clinical research, which holds the possibility to reduce the time and cost needed for clinical trials on COVID-19 and other diseases.

Methods: Using the PubMed database, we analyzed six relevant scientific articles regarding the possible application of in silico clinical trials in testing the therapeutic and investigational methods of managing different diseases.

Results: Successful use of in silico trials was observed in many of the reviewed evidence.

Conclusion: In silico clinical trials can be used in refining clinical trials for COVID-19 infection.

Keywords: in silico, clinical trials, COVID-19, SARS-CoV-2, vaccine

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1. Introduction

Computer simulations have been used in airplane and car design with great performance and accuracy for years [1, 2]. Recently, companies and researchers have started to pay more attention to the use of computational modeling in areas like fluid dynamics, ventricular septal deceives, and movement mechanics of hip implants [3, 4]. The general commissioner of the American Food and Drug Administration (FDA) in 2017 recognized computer simulation as a possible solution for approved medical instruments. Furthermore, in 2016, a guide for reporting Computational Modeling Studies in Medical Device Submissions was issued by the FDA to provide clear recommendations for organizing and submission of these studies [5].

Randomized clinical trials represent almost two-thirds of the approximately $2.6 billion needed to develop a new drug [6], it is a very long and expensive method for designing and validating new therapies and technologies with a very disturbing high failure rate [7]. Furthermore, real clinical trials may indicate a drug to be ineffective, however, it rarely indicates the reason behind this failure. This leads to the rejection of the drug despite the possibility of it working with small modifications [8]. From here, the possibility of other methods like in silico simulation with coherent abilities to provide fast and accurate results seems very promising especially in times of pandemic like the current COVID-19 where time is a very limited resource.

The term in silico clinical trial (ISCT) was first coined in 2011 by the Virtual Physiological Human (VPH) Institute which defined it as:

> *The use of individualized computer simulation in the development or regulatory evaluation of a medicinal product, medical device, or medical intervention. It is a subdomain of "in silico medicine, the discipline that encompasses the use of individualized computer simulations in all aspects of the prevention, diagnosis, prognostic assessment, and treatment of disease."*

Since then, the concept of changing clinical evidence with in silico evidence is slowly being more accepted, which was made evident by the FDA acceptance of in silico simulation as a possible replacement for animal studies in the assessment of artificial pancreas technologies [9, 10].

In silico trials can be used for the noninvasive assessment of medical conditions. For example, in vivo computed tomography data were used to create individualized models that accurately predict the changes in vertebrae bone strength in mice [11], and the incidence of spine and hip fracture in humans [12]. From these observations, we

contemplate that ISCTs can be used for choosing the specific mode of therapy with the appropriate doses for individual COVID-19 patients, depending on their individualized parameters like immunodeficiency and renal impairment. This will be a significant step in the path of individualized medicine. Furthermore, since this information can be available within hours to minutes to the clinical provider, this acts as a significant bonus when compared with the classical clinical trial.

This work aims to discuss the possible use of in silico trials in the design and testing of therapeutic and preventive measures to either reduce, refine, or partially replace human clinical trials done on the common disease with a focus on the possible applications of these methodologies in COVID-19 trials.

2. Materials and Methods

2.1. Types and phases of clinical trials

Clinical trials have been for a long time the golden tool for validating the efficiency of new drugs and biomedical products. It is a very long and complicated procedure containing five basic phases:

1. Preclinical phase: done in animals to understand the physiological action of the drug and four clinical trials in humans to assess the appropriate dose and duration of the drug and acquire data about short- and long-term side effects [13]. Figure 1 shows a schematic representation of the clinical trials pathway to further elucidate the relation between the different phases.

Although randomized clinical trials are the gold standard for evaluating the effectiveness and safety of the new medication and biomedical products, nonrandomized studies could still provide valuable and reliable input especially in fields such as forensic mental health where the randomized clinical trial may be inappropriate, therefore assisting in the decision for accepting or rejecting the new products or procedures [14].

2.2. COVID-19 conventional therapeutic clinical trials

In the past months since COVID-19 appeared from Wuhan, China, and spread around the world, over 3876 clinical studies have been recorded globally on clinical trial registries, including over 500 randomized controlled trials.

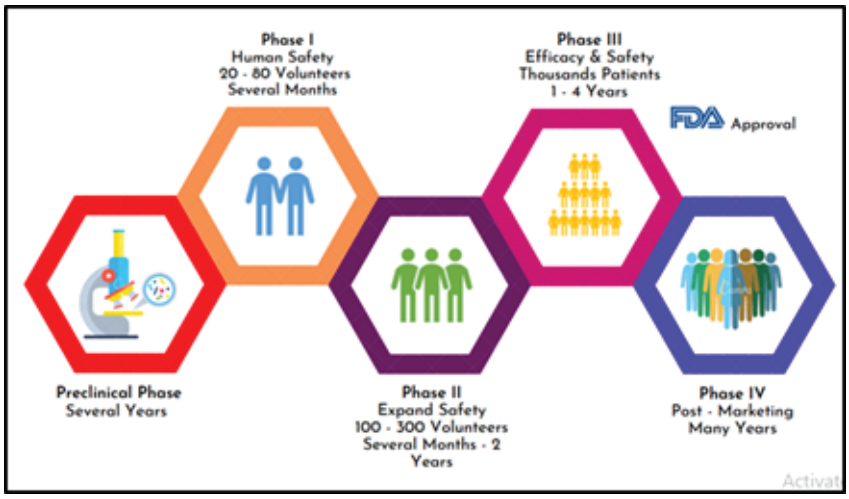

Figure 1: Preclinical and clinical trial phases. FDA: Food and Drug Administration (https://www.fda.gov/)

Such rapid development and inauguration of clinical trials are remarkable but presents challenges, including the possibility for replication and rivalry [15].

The most common therapeutic agent being trialed presently is hydroxychloroquine (261 trials with some trials planning to recruit over 25,000 participants), followed by antiinfective and antiparasitic agents as illustrated in Figure 2.

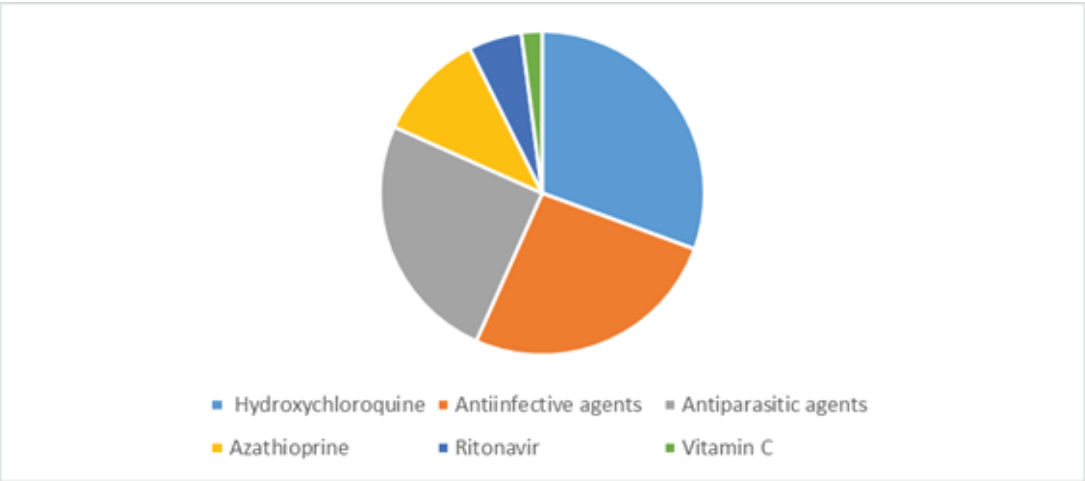

Figure 2: Distribution of the most common therapeutic clinical trials on COVID-19, as reported by clinicaltrials.gov.

Currently, there are many contestant drugs in preclinical and early phase development and these form a pipeline for future large clinical trials if current applicant therapies prove ineffective or unsafe [15].

With 15% of patients complaining of the severe form of the disease, hospitals being stunned worldwide, and a global mortality rate of 5.7%, new modes of therapy are instantly needed. New interventional clinical trials for COVID-19 treatment encompass the recycling of an old antiviral drug formerly used to treat the Ebola virus known as

Remdesivir or the combination of two antivirals: Ritonavir + Lopinavir, which has been used in the past to treat the HIV infection. Additionally, active clinical trials comprise the use of drugs approved for different therapeutic indications, as in the case of using monoclonal antibodies against interleukin-6 receptor (anti-IL-6R). This recycling strategy established on the reuse of approved drugs is commonly stated as drug repurposing and is largely effective, as verified by instances of repurposing treatments in cancer and other human diseases [16].

ISCTs could be very cost-effective in testing all possible therapeutic drugs for COVID-19 as it provides a fast and reliable method for acquiring data about the efficiency of these therapeutics with no or minimal human involvement.

2.3. COVID-19 vaccine clinical trials

The ideal design SARS-CoV-2 vaccine must address the need of vaccinating both the general population and high-risk individuals. Furthermore, the designed vaccines must adhere to the basic standard for vaccines like non-allergenicity, antigenicity, potency, and ability to induce both cellular and humoral response among others [17, 18].

At the time of writing this paper, and according to the WHO, there were 47 COVID-19 vaccine candidates around the world under clinical evaluation at different stages. Most of these (14 trials) were using a protein subunit platform. Furthermore, there are 155 trials currently under preclinical evaluation with the majority (55 trials) using protein subunit platforms [19]. Figure 3 illustrates the distribution of these vaccines.

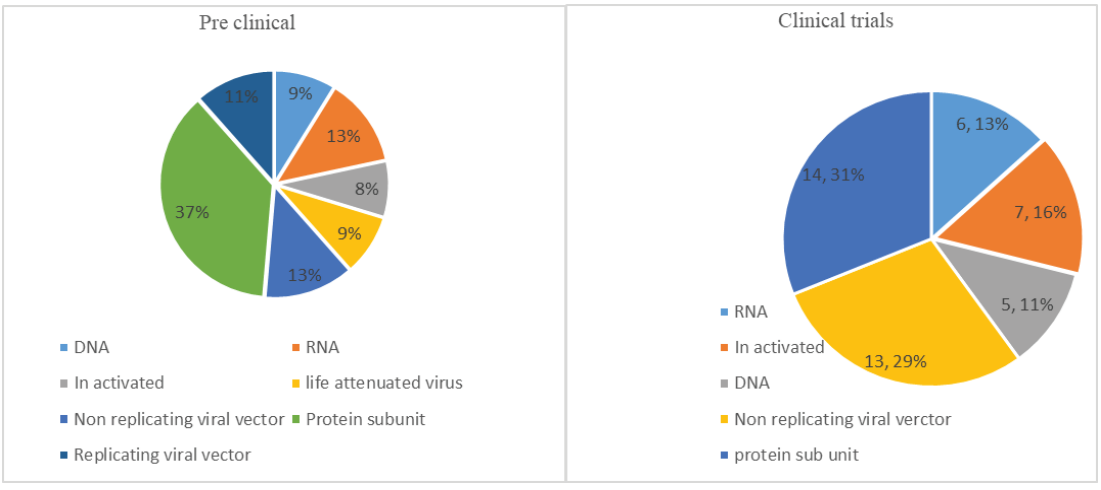

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Figure 3: (A) The number of COVID-19 vaccine candidates in preclinical evaluation trials. (B) The number of COVID-19 vaccines clinical trials in clinical evaluation.

2.4. Artificial intelligence and COVID-19

Artificial intelligence (AI) has been useful in designing drugs and vaccines against several organisms, including bacteria and viruses that are known to cause severe infections. In particular, AI has a great outlook on developing vaccines against diseases such as HIV and malaria that have been problematic [20–23]. The SARS-Cov-2 is a single-stranded, enveloped RNA virus which has several antigenic proteins: the matrix (M) protein, nucleocapsid (N) protein, envelope (E) protein, and spike (S) surface glycoprotein [24]. The S protein has two subunits S1 and S2, majorly responsible for the viral fusion and binding, respectively. It is the primary focus of the development of serology tests and vaccines for the disease. Understanding the complete sequence of the S glycoprotein has been explored to design a multi-epitope vaccine with VaxiJen, ToxinPred, and IEDB servers. This resultant in silico vaccine was shown to have an excellent ability to stimulate a robust immune response against the virus [25].

2.5. ISCTs

2.5.1. Earlier applications

The process of developing new biomedical products requires three steps: design, preclinical assessment, and clinical assessment; all of which can be enriched by the use of in silico simulations [10].

In silico trials can be used to refine clinical trials and reduce the number and duration of animals and humans involved in this experimentation. For example, when a surrogate measurement is used in the in silco trial, the reproducibility of the in vivo studies is highly improved, and hence the numbers required for statistical significance will be reduced, as illustrated in the study by Orwoll *et al.*, where bone fracture endpoint was replaced with a bone strength endpoint [26].

Another use is where individualized models can partially replace animals or humans in a trial. An example of this is the approval by the FDA of the complete replacement of experiments on animals (dogs) with a UVA/PADOVA diabetes type I simulator for the assessment of new artificial pancreas technologies [27].

2.5.2. Future applications

Regular use of in silico software may become the common theme in medical research, where simulation could be provided for a large number of virtual patients (<1000) in a matter of minutes to hours, with tools to validate and replicate these trials. Depending on the result of these in silico trials, new drugs and biomedical products can be approved by health agencies without the need for major human involvement in traditional randomized clinical trials. Table 1 summarizes the difference between ISCT and traditional clinical trials.

Table 1: Comparison between in silico and randomized clinical trials.

Types of trial	Cost	Duration	Human risk	Need for validation	Type of disease understudy
Traditional randomized clinical trial	Expensive	Several years	Low to moderate human risk	Current gold standard with no need for validation	Better in common disease and for detecting common side effects for drugs or procedures
In silico clinical trial	Relatively cheaper	Minutes to hours	No human risks	Currently need validation in most cases with subsequent small-size random clinical trials	Good for both common and rare diseases and rare side effects

2.6. Available ISCT software

2.6.1. Simulo

Simulo is a user-friendly clinical trial simulator developed by SGS Exprimo. It uses Monte Carlo simulations and R code to assess study designs and compare different dosing strategies using mixed-effects models. This will help in the optimization of the steps of the clinical trial and the prediction of the probability of success, the optimal dose, the cost-effectiveness, and finally to go or not to go decisions.

There are two available versions of the software – the basic free version and the expert version. While the basic version is suitable for small projects and demonstration purposes, the expert version provides more advanced tools like validation algorithms, which make it more acceptable for scientific publication (https://exprimo.com/simulo).

This software was used in 2018 in a study done by Murad Melhem and colleagues to model neutrophil response to granulocyte colony-stimulating factor (G-CSF) in patients

with chemotherapy-induced neutropenia [28]. The successful use of this software in this work is encouraging and shows that it can be useful in other diseases like the current COVID-19 disease.

2.7. Highly Efficient Clinical Trials Simulator (HECT)

HECT is a web-based clinical trial simulator for the planning of adaptive trials (a type of trial that is more flexible than conventional clinical trials), written with statistical software R, with a friendly user graphical interface. This simulator allows the user to set multiple clinical trial parameters and investigate different settings such as varying treatment effects, control response, and adherence. It is open-source and is most useful for clinical trial investigators who don't have the specialized statistical capacity or access to a commercial trial simulator. An important note here is that the simulation code in this software has been validated against six clinical trials designed by the designer of the software [29] (https://mtek.shinyapps.io/hect/).

2.8. Previous studies on ISCTs

In total, six ISCTs were retrieved from the PubMed database. These trials were used for common diseases such as breast cancer, diabetes, and tuberculosis. The first trial simulates a clinical trial of immunotherapies in metastatic breast cancer, which was carried out by Hanwen Wang and colleagues. In this study, a quantitative systems pharmacology model was built to integrate immune cancer cell interactions in patients with breast cancer, simulating central, peripheral, tumor-draining lymph node and tumor compartments. The proposed model provides a platform that can be further adapted to other types of immunotherapy which may contribute to the optimization of breast cancer treatment. The other in silico trial was carried out by Susan G. Hilsenbeck and C. Kent Osborne in breast cancer, where they identified the role of adjuvant tamoxifen in progesterone-positive breast cancer.

Regarding the non-small cell lung cancer, an ISCT was taken, comparing the photon and proton radiotherapy effect on this patient group, which showed a reduction of the integral dose (ID) and the dose to the Organ at Risk (OAR) with protons therapy instead of photons even with dose escalation. In addition, simulation of clinical trials was conducted to identify and individualize optimal isoniazid doses in children with tuberculosis, where they concluded that in children, isoniazid should be optimized based upon disease process, age, and acetylation status.

Additionally, another ISCT was done, this time testing bioactive substance effect on healthy smokers. In this study, the result could explain the synergistic action mechanisms of the Sanghuang–Danshen (SD) bioactive in the regulation of vascular endothelial dilation, confirming the SD potential effect in releasing the vascular stiffness and decreasing blood pressure in healthy smokers. Finally, an ISCT with the University of Virginia tested the use of inhaled insulin using Type I Diabetes Simulator which in this study provides superior postprandial control and smaller risks of hypoglycemic events [30–35]. Table 2 summarizes these trials.

Table 2: Summary of six ISCTs.

Authors	Num. of virtual patients	Disease understudy	Medication or device	Main outcome
Roberto Visentin and colleagues **[30]**	100	Type 1 diabetes militias	Inhaled technosphere insulin (Afrezza)	Relative to insulin lispro, postmeal dosing, or split dosing of inhaled insulin, in combination with an appropriate titration rule, can achieve superior postprandial glucose control.
Prakash M. Jeena and colleagues **[31]**	10,000	Tuberculosis in children	Isoniazid	None of the isoniazid routine doses (between 2.5 and 40 mg/kg/day) would achieve 80% effective concentration in most of the children (<90%).
Hanwen Wang and colleagues **[32]**	18	Metastatic breast cancer	Anti-CTLA-4 and anti-PD-L1 immunotherapies	In combination therapy, the reduction in tumor size is moderately enhanced, compared to the anti-PD-L1 monotherapy. Furthermore, the proposed model display the potential to make predictions of tumor response of individual patients when sufficient clinical measurements are provided.
Susan G. Hilsenbeck and C. Kent Osborne **[33]**	50,000	Progesterone receptor(–/+) breast cancer	Arimidex and Tamoxifen	In PR– cases, initial therapy with an aromatase inhibitor is superior to tamoxifen. In PR+ cases, tamoxifen is only modestly inferior to Arimidex at the outset, with a higher survival rate at 7.5 years.
Erik Roelofs and colleagues **[34]**	25	Non-small cell lung cancer	Photon and proton radiotherapy	When compared with photon, passive scattered conformal proton therapy (PSPT) will provide a larger dose to the tumor tissue with less damage to at-risk organs.
Yeni Lim and colleages **[35]**	72	Vascular stiffness in healthy smokers	Sanghuang–Danshen (SD) bioactive	Compared to a placebo alone, SD consumption at 900 mg/day for four weeks improves pulse wave velocity (p = 0.0497), and reduces systolic blood pressure, therefore reducing vascular stiffness in healthy smokers.

3. Results

Only six relevent ICTs were through PubMed. These trials were conducted for common diseases such as breast cancer, diabetes, and tuberculosis. The first trial simulateed a clinical trial of immunotherapies in metastatic breast cancer, which was carried out by Hanwen Wang and colleagues. In this study, a quantitative systems pharmacology model was built to integrate immune cancer cell interactions in patients with breast cancer, simulating central, peripheral, tumor-draining lymph node, and tumor compartments. The proposed model provides a platform that can be further adapted to other types of immunotherapy which may contribute to the optimization of breast cancer treatment. The other ICT was carried out by Susan G Hilsenbeck and C Kent Osborne in breast cancer, where they identified the role of adjuvant tamoxifen in progesterone-positive breast cancer. Table 2 summarizes the main aspects of the involved trials

4. Discussion

4.1. Validation of ISCTs

Validation of ISCTs is the procedure that evaluates the degree of how much the computer model and simulation agenda is capable of imitating a reality of interest. It is a necessary step before application in clinical studies. This step can be done either through comparison with existing literature or through standardizing the methodology used in these trials. Assessing these two factors will help in proving the model integrity and hence the reliability of the ISCT [36, 37].

4.2. Limitation of ISCTs

Resistance from the research professionals with limited interest in physics and mathematics, and the difficulty in simulating the complex physiological systems with the specialized advanced technical requirement to create new simulator software are the major hurdle of ISCT simulations.

4.3. Proposed ISCT protocol for COVID-19 vaccine

Building on the previous evidence, we believe ISCTs could be a plausible option in the current COVID-19 pandemic, as the software and models needed can be tested and validated against the preliminary results of the current COVID-19 clinical trials.

Specially designed models or an already available one like the model designed by Renz *et al.* simulating human alveolar macrophage with SARS-CoV-2 [38] can be used as a basis for the in silico trials. Furthermore, the Universal Immune System Simulator (UISS) platform which is an agent-based simulator is suggested by Giulia Russo and colleagues as a good choice for vaccine design studies [39]. Other platforms like Simulo and HECT can also be used in the current COVID-19 ISCTs with the following suggested parameters:

1. Type of trials: adaptive randomized ISCTs.
2. Number of patients: 10,000 virtual patients, between 6 and 59 years old who will receive two dosages of the vaccine or placebo.
3. Dataset: data for comparison and validation can be retrieved from open online databases like UK datasevice or specialized research centers.
4. Duration: 5 years.
5. Drug: multi-epitope peptide vaccine using spike protein as an immunogenic target.
6. Primary end target results: number of patients with positive and negative RT-PCR, death rate.
7. Secondary end target results: number of patients with documented allergies or vaccine side effects.

4.4. Study limitation

The lack of standardized systemic review with appropriate statistical measurement in this work may undermine the acquired results and conclusion, however, the significance of this conclusion should not be overlooked.

5. Conclusion

ISCTs can truly transform the war against the COVID-19 pandemic. It is an attractive and applicable tool to accelerate the rate of approval for new therapeutics and vaccines for

common and rare diseases. Furthermore, it is probably the most cost-effective method to reduce, refine, and partially replace COVID-19 conventional clinical trials ensuring both relevant results and minimal human risks.

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Ethical Considerations

Not required.

Competing Interests

The authors declare that they have no conflict of interest regarding this paper.

Availability of Data and Material

All relevant data of this study are available to any interested researchers upon reasonable request to the corresponding author.

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References

[1] Krus, P., Braun, R., Nordin, P., et al. (2012). Aircraft system simulation for preliminary design. Paper presented at the 28th International Congress of the Aeronautical Sciences. Retrieved from: https://www.researchgate.net/publication/233919742_Aircraft_System_Simulation_for_Preliminary_Design

[2] Janoško, I., Polonec, T., Kuchar, P., et al. (2017). Computer simulation of car aerodynamic properties. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 65, no. 5, pp. 1505–1514.

[3] Geringer, J., Imbert, L., and Kim, K. (Eds.). (2014). Computational modeling of hip implants. In *Computational modelling of biomechanics and biotribology in the musculoskeletal system* (pp. 389–416). Sawston, United Kingdom: Woodhead Publishing.

[4] Wong, K. K. L., Wang, D., Ko, J. K. L., et al. (2017). Computational medical imaging and hemodynamics framework for functional analysis and assessment of cardiovascular structures. *BioMedical Engineering OnLine*, vol. 16, article 35.

[5] Food and Drug Administration. (2016). *Reporting of computational modeling studies in medical device submissions* (Docket No. FDA-2013-D-1530). Retrieved from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/reporting-computational-modeling-studies-medical-device-submissions

[6] DiMasi, J., Grabowski, H., and Hansen, R. (2015). The cost of drug development. *The New England Journal of Medicine,* vol. 372, p. 1972.

[7] Wong, C., Siah, K., and Lo, A. (2018). Estimation of clinical trial success rates and related parameters. *Biostatistics*, vol. 20, no. 2, pp. 273–286.

[8] Mehta, D., Jackson, R., Paul, G., et al. (2017). Why do trials for Alzheimer's disease drugs keep failing? A discontinued drug perspective for 2010-2015. *Expert Opinion on Investigational Drugs*, vol. 26, no. 6, pp. 735–739.

[9] Viceconti, M., Cobelli, C., Haddad, T., et al. (2017). In silico assessment of biomedical products: the conundrum of rare but not so rare events in two case studies. *Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine*, vol. 231, pp. 455–466.

[10] Viceconti, M., Henney, A., and Morley-Fletcher, E. (2016). In silico clinical trials: how computer simulation will transform the biomedical industry. *International Journal of Clinical Trials*, vol. 3, no. 2.

[11] Nyman, J., Uppuganti, S., Makowski, A., et al. (2015). Predicting mouse vertebra strength with micro-computed tomography-derived finite element analysis. *BoneKEy Reports*, vol. 4, p. 664.

[12] Kong, S. H., Ahn, D., Kim, B. R., et al. (2020). A novel fracture prediction model using machine learning in a community-based cohort. *JBMR Plus*, vol. 4, no. 3, e10337.

[13] Umscheid, C. A., Margolis, D. J., and Grossman, C. E. (2011). Key concepts of clinical trials: a narrative review. *Postgraduate Medicine*, vol. 123, pp. 194–204.

[14] Ferriter, M. and Huband, N. (2005). Does the non-randomized controlled study have a place in the systematic review? A pilot study. *Criminal Behaviour and Mental Health: CBMH*, vol. 15, pp. 111–120.

[15] Davis, J. S., Ferreira, D., Denholm, J. T., et al. (2020). Clinical trials for the prevention and treatment of coronavirus disease 2019 (COVID-19): the current state of play. *The Medical Journal of Australia*, preprint.

[16] Ciliberto, G. and Cardone, L. (2020). Boosting the arsenal against COVID-19 through computational drug repurposing. *Drug Discovery Today*, vol. 25, no. 6, pp. 946–948.

[17] Ma, C., Wang, L., Tao, X., et al. (2014). Searching for an ideal vaccine candidate among different MERS coronavirus receptor-binding fragments–the importance of immunofocusing in subunit vaccine design. *Vaccine*, vol. 32, pp. 6170–6176.

[18] Six, A., Bellier, B., Thomas-Vaslin, V., et al. (2012). Systems biology in vaccine design. *Microbial Biotechnology*, vol. 5, pp. 295–304.

[19] WHO. (2021). Draft landscape of COVID-19 candidate vaccines. Retrieved from: https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines

[20] Hessler, G. and Baringhaus, K. H. (2018). Artificial intelligence in drug design. *Molecules*, vol. 23, no. 10, p. 2520.

[21] Yu, W. H., Zhao, P., Draghi, M., et al. (2018). Exploiting glycan topography for computational design of Env glycoprotein antigenicity. *PLOS Computational Biology*, vol. 14, e1006093.

[22] Goodswen, S. J., Kennedy, P. J., and Ellis, J. T. (2014). Vacceed: a high-throughput in silico vaccine candidate discovery pipeline for eukaryotic pathogens based on reverse vaccinology. *Bioinformatics*, vol. 30, pp. 2381–2383.

[23] Abdelmageed, M. I., Abdelmoneim, A. H., Mustafa, M. I., et al. (2020). Design of a multiepitope-based peptide vaccine against the E protein of human COVID-19: an immunoinformatics approach. *BioMed Research International*, vol. 2020, 2683286.

[24] Tang, Y. W., Schmitz, J. E., Persing, D. H., et al. (2020). Laboratory diagnosis of COVID-19: current issues and challenges. *Journal of Clinical Microbiology*, vol. 58, no. 6, e00512–20.

[25] Abraham Peele, K., Srihansa, T., Krupanidhi, S., et al. (2020). Design of multi-epitope vaccine candidate against SARS-CoV-2: a in-silico study. *Journal of Biomolecular Structure & Dynamics*, vol. 39, no. 10, pp. 3793–3801.

[26] Orwoll, E. S., Marshall, L. M., Nielson, C. M., et al. (2009). Finite element analysis of the proximal femur and hip fracture risk in older men. *Journal of Bone and Mineral Research*, vol. 24, pp. 475–483.

[27] Man, C. D., Micheletto, F., Lv, D., et al. (2014). The UVA/PADOVA type 1 diabetes simulator: new features. *Journal of Diabetes Science and Technology*, vol. 8, no. 1, pp. 26–34.

[28] Melhem, M., Delor, I., Pérez-Ruixo, J. J., et al. (2018). Pharmacokinetic–pharmacodynamic modelling of neutrophil response to G-CSF in healthy subjects and patients with chemotherapy-induced neutropenia. *British Journal of Clinical Pharmacology*, vol. 84, pp. 911–925.

[29] Thorlund, K., Golchi, S., Haggstrom, J., et al. (2019). Highly Efficient Clinical Trials Simulator (HECT): software application for planning and simulating platform adaptive trials [version 2; peer review: 2 approved, 2 approved with reservations]. *Gates Open Research*, vol. 3, p. 780.

[30] Visentin, R., Giegerich, C., Jäger, R., et al. (2016). Improving efficacy of inhaled technosphere insulin (Afrezza) by postmeal dosing: in-silico clinical trial with the University of Virginia/Padova type 1 diabetes simulator. *Diabetes Technology & Therapeutics*, vol. 18, pp. 574–585.

[31] Jeena, P. M., Bishai, W. R., Pasipanodya, J. G., et al. (2011). In silico children and the glass mouse model: clinical trial simulations to identify and individualize optimal isoniazid doses in children with tuberculosis. *Antimicrobial Agents and Chemotherapy*, vol. 55, no. 2, pp. 539–545.

[32] Wang, H., Milberg, O., Bartelink, I. H., et al. (2019). *In silico* simulation of a clinical trial with anti-CTLA-4 and anti-PD-L1 immunotherapies in metastatic breast cancer using a systems pharmacology model. *Royal Society Open Science*, vol. 6, no. 5, 190366.

[33] Hilsenbeck, S. G. and Osborne, C. K. (2006). Is there a role for adjuvant tamoxifen in progesterone receptor-positive breast cancer? An in silico clinical trial. *Clinical Cancer Research*, vol. 12, pp. 1049s–1055s.

[34] Roelofs, E., Engelsman, M., Rasch, C., et al. (2012). Results of a multicentric in silico clinical trial (ROCOCO): comparing radiotherapy with photons and protons for non-small cell lung cancer. *Journal of Thoracic Oncology*, vol. 7, pp. 165–176.

[35] Lim, Y., Song, T. J., Hwang, W., et al. (2019). Synergistic effects of sanghuang¯danshen bioactives on arterial stiffness in a randomized clinical trial of healthy smokers: an integrative approach to in silico network analysis. *Nutrients*, vol. 11, no. 1, p. 108.

[36] Rodrigues, L., de Lima, I., Ricardo Albano dos Santos, L., et al. (2018). Towards a standardized protocol for conducting randomized clinical trial for software. *Procedia Computer Science*, vol. 138, pp. 125–130.

[37] Viceconti, M., Pappalardo, F., Rodriguez, B., et al. (2020). In silico trials: verification, validation and uncertainty quantification of predictive models used in the regulatory evaluation of biomedical products. *Methods*, vol. 185, no. 1, pp. 120–127.

[38] Renz, A., Widerspick, L., and Dräger, A. (2020). FBA reveals guanylate kinase as a potential target for antiviral therapies against SARS-CoV-2. *Bioinformatics*, vol. 36,

no. 2, pp. i813–i821.

[39] Russo, G., Pennisi, M., Viceconti, M., et al. (2020). In silico trial to test COVID-19 candidate vaccines: a case study with UISS platform. *BMC Bioinformatics*, vol. 21, no. 17, article 527.