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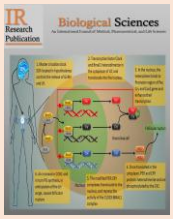

Emerging Human-Based Alternative Technologies as Replacements for Preclinical Animal Testing

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ABSTRACT

This review aims to explore recent advancements in human-based methodologies that are reshaping the landscape of preclinical research. It focuses on evaluating the scientific validity, translational relevance, and regulatory recognition of emerging non-animal platforms. By analyzing these approaches, the article seeks to demonstrate their potential in enhancing predictive accuracy, ethical responsibility, and the overall efficiency of biomedical research and drug development. Most predominantly used methods include Artificial intelligence models, in-vitro models, in-silico models, organ-on-chip systems, and other innovative technologies. Human organoids-on-chips (OrgOCs) combine human organoids (HOs) technology and microfluidic organs-on-chips (OOCs). HOs are related to biological analysis and genetic manipulation while OOCs can simulate external characteristics of organs like living tissue, OrgOCs served as 3D organotypic living models allowing them to recapitulate critical tissue-specific properties and predict human responses. Virtual screening, molecular docking, QSAR modeling, AI/ML based clinical computational models are some of the tools used in building non-animal modelling. Animal testing requirement of FDA will be replaced using above range of approaches in a laboratory setting. Implementation of the regimen shall begin for investigational new drug (IND) applications, where inclusion of NAMs data is encouraged, as outlined in road map guidance document12. In the future, computational approaches have the potential to catapult us into the realm of customized treatment, where individual differences are methodically examined leading to transformed drug development. Virtual screening and computer-based trials are emerging as ways to speed up drug development while reducing expenses. is critical to balance innovation with ethical data handling. In essence, the future of drug design is being charted by the dynamic interplay of computational prowess and biological insight, heralding a new era of targeted, efficient, and personalized therapeutics.

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Introduction

Traditionally, drug development broadly consists of Pre-discovery stages. Next is drug discovery stage, where scientists research drug molecules with potential therapeutic compounds or the therapeutic strategies that can either treat the disease itself or the symptoms. Further, proceeding with preclinical development stage that mainly focuses on determining the mode of action of the drug molecules while investigating the toxicity. Validating the efficacy in different in-vitro and in-vivo models and formulation is carried out during this stage. Once pre-clinical evaluations are established, overall testing of drug molecules in humans is conducted at the clinic stage.

In research finding a new treatment is always challenging. Although understanding biological systems and the development of associated technologies has progressed, the overall drug development process is still tedious and expensive. Latest approaches, like artificial intelligence and new in-vitro technologies are being used in to rationalize R&D to bring new drugs to patients effectively. However, several challenges remain (1).

Every year only a few new drugs are licensed for use, while there will be many candidate drugs that doesn't get through. It takes 12 years and over a billion for the R&D journey of the new drugs that make it to market. Only upon understanding of disease mechanisms and targets for new treatments can be identified.

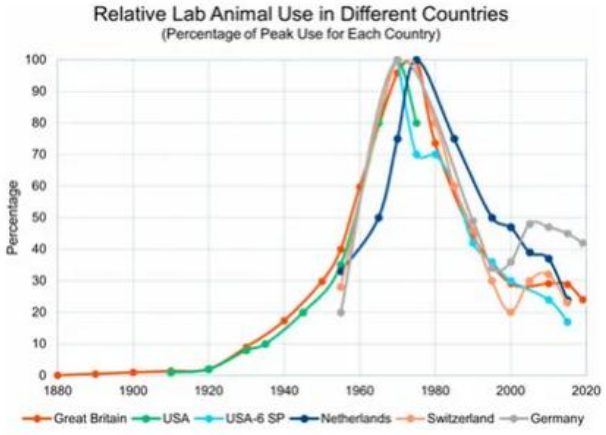

Figure 1. This figure, kindly provided by Dr. Andrew Rowan, shows that all animal use peaked in the 1970s in most industrialized countries. Taken from Wellbeing International (WBI) newsletter 31 December 2021 with permission.

On identifying the target, a potential therapeutic compound that acts on this target is identified. The most important stage is to conclude that these molecular compounds show safe effect. Before administering any molecules to humans, safety and efficacy tests are performed using different models, cells and animals. Only 50% of candidates make it through this stage and only a few from the initially selected compounds can be tested in humans. To test in UK, authorization by the Medicines and Healthcare products Regulatory Agency (MHRA) is essential before any drug testing in humans can be planned. The research/industrial body will put in a clinical trial application (CTA), which will be reviewed by both medical and scientific experts concluding that enough preliminary research has been done to allow validation in humans to kick start (2).

Global Current Practice

Drug development happens globally, and multi-regional clinical trial (MRCT) for regulatory submission has largely been conducted by several research based multi-national pharmaceutical companies with an aim to bring down the delay in time to launch in the markets and increase patient access to new as well as quality treatments. Sponsors face many challenges while conducting multiregional clinical trials and those challenges differentiated under categories like statistics, clinical, regulatory operational, and ethics. Regulatory bodies in different countries like USA, UK, Japan, and China have issued guidance documents about MRCT's. International conference on harmonization (ICH) has initiated the practice of following a harmonized guidance document on MRCT. In 2017 This guidance document is issued.3 Regulatory authorities (PMDA) in Japan have implemented measures to encourage the practice of MRCT to improve patient access and reduce the delay in bringing a drug to market. China Regulatory agency (NMPA) has also released a guidance document on MRCT. Drugs Controller General of India (DCGI), India may consider having a comprehensive guidance document for MRCT to lead upper age restriction of patients recruited for research to ensure uniformity in the universal study protocol (3).

Competing goals persist in ensuring safety and efficacy while rapidly delivering innovative therapies rapidly following regulatory processes. The United States depends on a strictly centralized process through one regulatory body, the Food and Drug Administration (FDA) while the European Commission harmonized regulations of 28 different countries to create European Union. The FDA has evolved as a consumer protection agency, while the European Commission adapted the need to harmonize inter-state commercial interests by preserving national "autonomy" (4).

While regulatory harmonization aims to facilitate MRCT, clinical trial success ultimately is contingent

upon the reliability and relevance of the preclinical models used to evaluate drug safety and efficacy.

Reliability of Animal Models in Predicting Human Outcomes

Recognizing the strengths and limitations of animal models is crucial and serve as a basis for decisions in global drug development.

Current limitations of traditional animal model trials

In pharmaceutical research animal testing is used to assess toxicity, however research shows that animal testing poorly predicts safety of drug molecules in

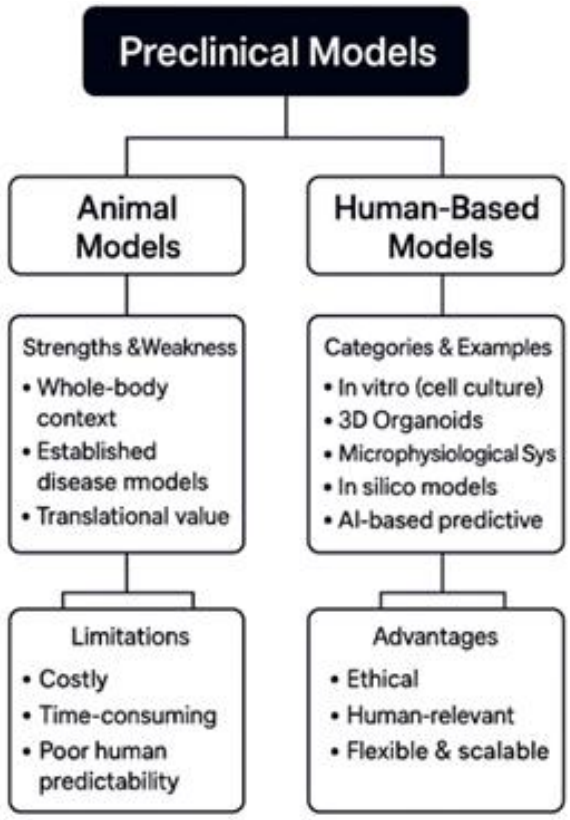

Figure 2. Schematic representation of innovative human-based preclinical models.

humans. Several human subjects were harmed in the clinical validation of drugs that were assumed safe during animal studies. Moreover, investigators are re-considering the scientific advantages of animal research. Pharmaceutical organizations and governing regulatory bodies are considering alternative methods to replace animal testing and hasten the drug development process there by safely delivering the new therapeutics for human use (5).

Challenges in animal experiments have gone beyond ethics as they are resource-intensive, expensive, time-consuming, and show limited judgement in humans' safety. This demonstrates the high failure rate in clinical trials of these potential therapeutic compounds due to issues like lack of efficacy or safety in human testing. These challenges also include "reproducibility of results" in R&D. Developing new drug is tedious and before a drug can be in the market, it must pass through preclinical studies in cells and animals followed by three phases of human clinical trials that include healthy volunteers, patients who vary from healthy group, and a large patient trial to prove the beneficial effect concluding that the drug is safe and effective for the intended use in the suffering individuals. Animal testing remain necessary until better techniques are assessed and accepted. Below figure 1 shows how the use of animals testing increased in the 1970s, for drug development. Also shows that other methods have been introduced to replace animal testing. Growing concerns related to animal ethics lead to questioning the use of animal testing and justification of animal suffering for drug advancement animal welfare while significantly supporting for developing alternatives (6).

Considering the human relatable characteristics, close comparison to human genes many animal species like primates, rodents, dogs, birds, ruminants, pigs were used in animal models and have played important role in several biomedical research fields like neuroscience and neurobiology, pharmacology and toxicology developing drug elements to multiple human conditions, both in the form of vaccines and new drugs. Even though animal models have been important for the research of global human health ethical questions still come up. Animal experimentation performed in compliance with government legislation, which reinstates the regard for animal welfare. Alternative models have been put in place, which can be used under controlled conditions allowing the implementation of the 'replacement' rule (7). Animal models have historically played a critical role in advancing biomedical research whether it is to understand disease mechanisms, evaluating and developing drug candidates. Their use has delivered essential preclinical evidence that has guided translation from preclinical to First-in-human (FIH) studies, despite challenges in extrapolating findings to humans precisely. Figure 2. Schematic representation of innovative human-based preclinical models. All models discussed in this figure are cited in Table 1, highlighting ethical and physiologically relevant alternatives to traditional animal testing.

Industry Shift to Virtual Patient Based Computational Modelling

Table 1. Advantages and disadvantages of animal and non-animal models.

Models	Advantages	Disadvantages	References
Animal models	Essential tools for preclinical drug screening; represent whole-body mechanisms and reactions; allow the identification of behavioural changes; many disease models have already been studied and are well established.	Not all biological responses can be translated to humans; spontaneous flares of disease cannot be represented; costly and not time-efficient; genetic variability is limited (majority of animals are inbred).	(8-12)
In vitro models	Able to target specific mechanisms.	Not all clinical symptoms can be replicated; not able to represent aging and life stages; identification of underlying mechanisms may be hindered.	(13-15)
Cell cultures	Less time-consuming and less expensive; allow screening of drug toxicity.	Difficulty replicating disease models; complexity of human system is not represented; disease progression cannot be assessed.	(16, 17)
3D models and organoids	Can accurately reproduce several critical aspects of the human tissues.	Small size with restricted viability; lack immune and vascular cells; costly and time-consuming.	(18, 19)
Micro physiologic systems	Closely mimic tissue microenvironments; allow the study of interactions between various cell types.	Isolated from other organ systems (restricts understanding of inter-organ effects); do not include an immune system; genetic uniformity (experimental bias).	(20, 21)
Invertebrate models	Brief life cycle; small size and simple anatomy; mouldable genetics; cost-effective (suitable for mass culture); Reduced ethical concerns.	Undeveloped organ systems; lack adaptive immune systems; no homologous human counterparts.	(22)
In silico models	Time-effective and low cost; absence of ethical considerations.	Require training based on previous data; unable to accurately replicate in vivo conditions.	(23)
Physiologically based pharmacokinetic	Can quantitatively describes and predicts drug concentration-time profiles; create a mechanistic representation of the drug in biological systems based on drug-specific data.	Not able to represent the complexity of in vivo transport mechanisms (numerous 'parameters' are not adequately represented).	(24)
Quantitative structure–activity relationship	Able to achieve a virtual screening of numerous chemical structures; efficient and cost-effective; may quickly identify initial drug candidates.	Often fail during the model-building phase.	(25)
Molecular docking	Widely used to assist different tasks of drug discovery programmes; allow protein-ligand, protein–protein, protein–peptide or nucleic acid–ligand docking; enable research of different molecular targets in various disease conditions; able to employ flexible algorithms in the calculations.	Limited in the calculation of accurate binding energies; lack suitable scoring function and searching algorithm; poor ability to efficiently combine both accuracy and speed.	(26, 27)
AI learning	May enhance disease monitoring; can aid in early detection, diagnostic accuracy and discovery of new treatments; able to conduct precise measurements of immunohistochemical biomarkers, cell counting and tissue properties analysis.	Still fairly undeveloped; may be difficult to obtain sufficient data for accurate training.	(28, 29)

Computational modelling is being adapted both for analysing biological systems and in clinical research for drug development. Research attention has shifted from creating a universal model of human to models like digital twins or a human representative model of clinical populations to use in silico clinical trials. Assessing disease-specific parameters, inter and intra individual differences, probability quantification, standardizing the quality-controlled data are key issues today, which calls for the need of open tools, data and metadata standards. The quantitative, biological and physiological and highly controlled methods provided by in-silico methods have become an integral part of medical research.

In silico methods have the key ability to hasten future progress also in the fields of integrated multi-physics modelling, multi-scale models (30).

Adapting Artificial intelligence methods to analyse physiology and disease mechanisms in generating new hypotheses there by assessing the treatment method can contribute to the evolution of preventive, diagnostic and therapeutic approaches. The correlation between mechanistic and data-driven methods may become a powerful force to lead the next era of AI applications, importantly where the AI algorithms can incorporate constraints from both physiological datasets and from multi-scale bond graph models that conserve mass, charge and energy (30).

Multiple challenges have been faced by the pharmaceutical and biological industry like the delay in drug development time, high failure rates in new drugs innovations there by failing to suffice for the robust data demands of regulatory bodies. A new solution to overcome these challenges is to make use of virtual patient groups to simulate drug effects in computer models where virtual patient subjects and predictive computational methods can serve as alternative option in drug development and diverse methodologies. Virtual patients can be created as digital twins via statistical inference to real time human or by randomly setting disease parameters across all clinical phases of drug development. The main advantages of virtual patient groups include potential cost savings and development success and increased innovation. Moreover, they offer improved representation of patient groups often marginalized in drug development efforts. By creating realistic virtual patients, more efficient and personalized drug development methods can be explored (31).

Computational medicine promises major advancements in biology and healthcare via physics-based simulations and AI to optimize disease diagnosis, personalize treatment strategies and accelerate medical innovation. Several computational models of human pathophysiology and medical treatments are being generated with latest applications in areas like cardiovascular diseases, orthopedics and cancer diagnosis. European initiatives such as the European Health Data Space and the Virtual Human Twins Initiative, aimed at fostering the development and application of computational medicine in healthcare (32).

Implementation of Industry Guidance Approaches

In 2022 President Biden signed into law the FDA Modernization Act 2.0. The bill importantly considers denying the Federal Food, Drug, and Cosmetics Act of 1938, which mandated animal testing for every new drug development protocol (33).

U.S. FDA took groundbreaking step to progress drug development by replacing animal testing in the development of monoclonal antibody therapies and medicinal compounds with more effective, human-relevant methods. The method is designed to improve drug safety and improve the assessment process, while reducing animal experimentation, decreasing costs spent in research and development (R&D) costs and over all drug prices (34).

Animal testing requirement of FDA will be replaced using a range of approaches, including AI-based computational models of toxicity and cell lines and organoid toxicity testing in a laboratory setting (so-called NAMs data). Implementation of the regimen shall begin for investigational new drug (IND) applications, where inclusion of NAMs data is encouraged, as outlined in road map guidance document (34).

Working in close partnership with federal agencies such as the National Institutes of Health, the National Toxicology Program and the Department of Veterans Affairs, the FDA aims to accelerate the validation and adoption of these innovative methods through the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) (34).

In efforts to promote human-relevant testing strategies worldwide, regulatory agencies such as European Medicines Agency (EMA) have endorsed the new approach methodologies (NAMs) in regulatory submissions. World Health Organization (WHO) promotes global harmonization of non-animal testing strategies and develop guidance on non-animal testing and the 3Rs to decrease animal experimentation, while Organization for Economic Co-operation and Development (OECD) delivers guidance on use of validated alternative methods for assessing chemical safety testing.

Required measures required for the shift

Legislation currently permits alternatives to animal testing, including human iPSC-based assays (iPSCs), organoids, organs-on-chips (OoCs), and AI-driven methods such as generative adversarial networks (GANs) (35). The FDA Modernization Act 2.0 promotes these approaches to strengthen preclinical research (35). Human-relevant NAMs, including advanced in vitro systems and computational models, can improve the prediction

Table 2. Comparison of AI, human-based lab models, and in silico clinical trials in drug development.

Methodology	Key Advantage/s	Key Challenge/s	Validated Performance	References
AI-Based Computational Models (Section 6a)	High accuracy in toxicity prediction; rapid and scalable screening; adaptive learning with new data	Data scarcity; model interpretability; multi-source integration; regulatory acceptance	Acute toxicity prediction: 85–95% accuracy; Organ-specific toxicity: 80–90%	(37)
Human-Based Lab Models (Organoids & Organs-on-Chips) (Section 6b)	Physiologically relevant; recapitulates tissue-specific functions; supports personalized medicine; accelerates preclinical-to-clinical translation	Fully vascularized/immune organoids remain challenging; scalability; cost	PK/PD prediction: in vivo-like; Patient-derived organoids allow optimized drug dosing	(38)
AI-Enabled In Silico Clinical Trials (6b)	Virtual patient cohorts; increases case group size; trial design optimization; predictive of trial success	ISCT credibility; hybrid model validation; clinical outcome mapping; adequacy assessment	Virtual cohort size can match/exceed real trials; Predicts device/drug safety & effectiveness; Hierarchical validation supported	(39, 40)

of pharmacokinetics, efficacy, and toxicity profiles, addressing translational gaps between preclinical studies and clinical outcomes.36 FDA and EMA are supporting the implementation of these innovative methods as the standard in early-stage drug development (36).

Aligned with the evolving regulatory landscape, innovative computational and human-relevant approaches, such as AI-based models and organoid systems, are being integrated into preclinical and clinical drug research.

New approaches and Methodologies

AI based computational models of toxicity

AI technology like machine learning and deep learning algorithms has robust data processing and pattern recognizing abilities where large amount of data of drug can be assessed for both drug activity and toxicity with high precision.

While high costs, ethical concerns, and cross-species variability can be answered major challenges around AI models persist that includes data scarcity, model interpretability and multi-source data integration and hence addressing these gaps stands important in continual development of robust, interpretable ML models underpinned by standardized evaluation metrics (37).

Human-based lab models (organoids and organs-on-a-chip)

OrgOCs combine human organoids (HOs) and microfluidic organs-on-chips (OOCs). HOs enable biological analysis and genetic manipulation, while OOCs simulate organ-level physiology. Together, OrgOCs serve as 3D organotypic models that recapitulate tissue-specific properties and predict human responses. Organoid chips have been developed for intestine, brain, kidney, and liver, reproducing in vivo-like pharmacokinetics and disease responses, thus reducing reliance on animal studies (38). They also support wound healing, tissue regeneration, and applications in cell and gene therapy, accelerating preclinical-to-clinical translation. Challenges remain in forming fully vascularized or immune organoids (38).

In silico clinical trials (ISCTs) are digital simulations of human trials, enabling virtual cohorts, trial optimization, and prediction of trial outcomes (39). ISCTs often combine multiple sub-models (physics-based, data-driven, rule-based) representing patients, devices, and interventions, translating simulation outputs into clinical outcomes (36,38). Credibility of ISCTs requires hierarchical validation of sub-models, model risk assessment using decision trees, and evidence gathering for clinical outcome mapping (40-42). Major challenges in implementing ISCTs include evaluating hybrid models that integrate physics-based and AI-driven components (36, 37), establishing clinical outcome mapping models to translate simulation outputs into

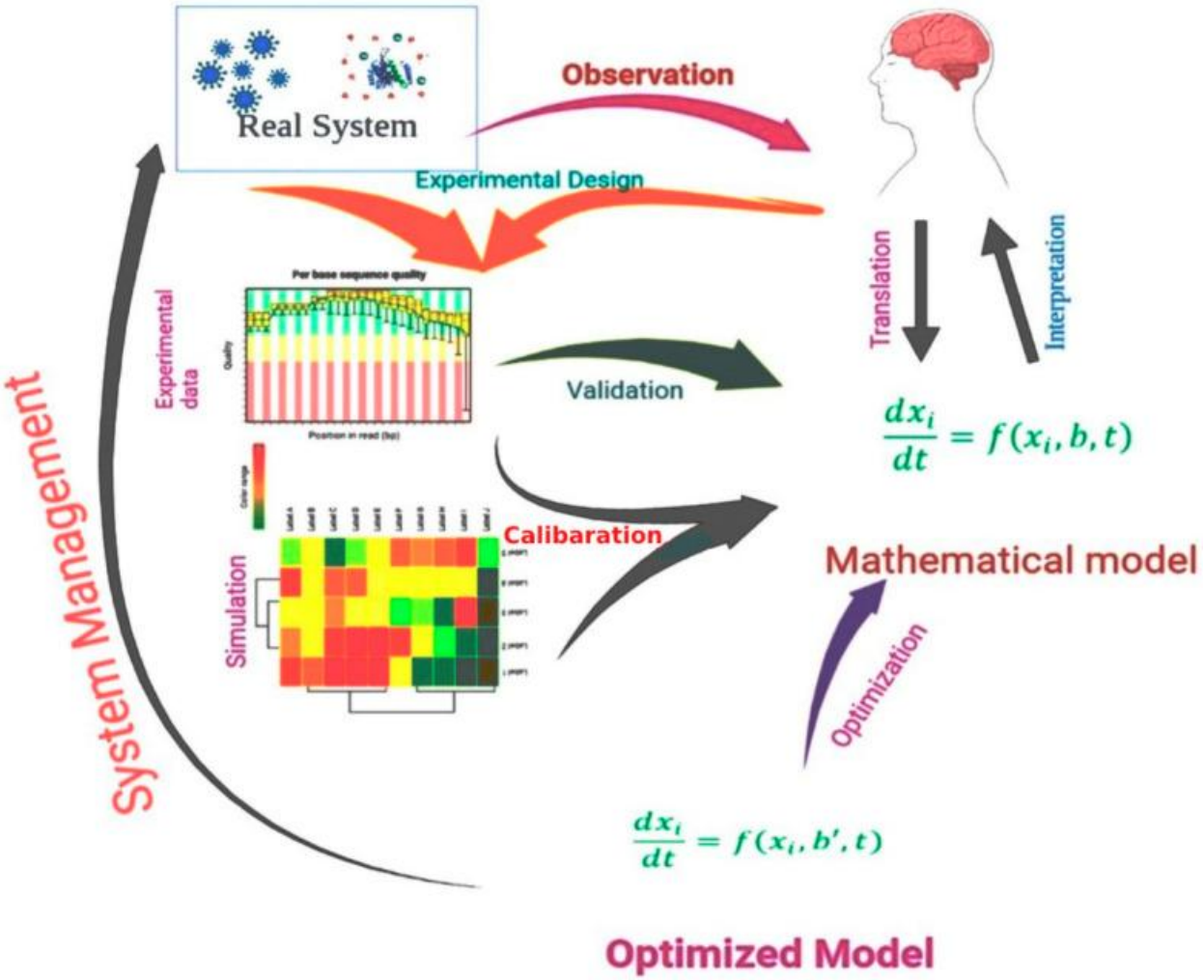

Figure 3. A schematic representation of a mathematical model, including experimental design, experimental data analysis, model optimization, and model validation, used in modern drug design approaches (56).

patient-relevant endpoints (42), defining credibility factors for non-traditional evidence sources (36,37,43-45), and assessing adequacy to ensure that all clinical trial endpoints are addressed (46). Addressing these challenges is critical for realizing the full potential of ISCTs in supplementing or replacing traditional clinical trials (46).

Successful Regulatory Submissions

Virtual screening, computer-based technique to identify potential drug candidates as part of drug discovery. Pharmacophores map the essential features for drug activity initially by considering physicochemical properties of the drug.

Catalyst, Ligand Scout, GRID, or LUDI are some of the well-known ligand or protein derived phramacophores. For comparative analysis and identifying similar compounds, fast search methods including CATS and feature trees are used (47).

Challenges and Opportunities

Although computational tumor models hold considerable promise, their broader adoption has been limited by difficulties in validation due to a lack of high quality longitudinal datasets and the trade off between biological fidelity and computational cost. Integrating diverse data types such as omics, imaging, and clinical records remains challenging, and developing models that reflect underlying biological mechanisms requires interdisciplinary expertise and careful hypothesis formation. Advances in machine learning and artificial intelligence are enhancing adaptability and predictive accuracy, and hybrid frameworks that combine mechanistic and data driven approaches are increasingly feasible, with techniques such as surrogate modeling, symbolic regression, and physics informed neural networks helping to improve model calibration and discovery. The emergence of patient specific “digital twins” further promises clinical utility by simulating disease progression and treatment responses, though

Table 3. List of docking tools, their advantages, and disadvantages (52).

Tool	Application	Advantages	Disadvantages
AutoDock Vina	Predicting the binding affinities and orientations of ligands.	Fast, accurate, and easy to use.	May not be as accurate for complex systems.
AutoDock GOLD	Predicting the binding affinities and orientations of ligands, especially for flexible ligands.	Accurate for flexible ligands.	Requires a license and can be expensive.
Glide	Predicting the binding affinities and orientations of ligands.	Accurate and integrated with other Schrödinger tools.	Requires the Schrödinger suite, which can be expensive.
DOCK	Predicting the binding affinities and orientations of ligands and performing virtual screening.	It is versatile and can be used for both docking and virtual screening.	Can be slower than other tools.
LigandFit	Predicting the binding affinities and orientations of ligands.	Easy to use and integrated with other Schrödinger tools.	May not be as accurate for complex systems.
SwissDock	Predicting the binding affinities and orientations of ligands.	Easy to use and accessible online.	May not be as accurate for complex systems.

issues of data privacy and model maintenance persist. Sustained progress depends on interdisciplinary collaboration, high quality experimental and clinical data, and continued advances in AI methodologies to generate biologically relevant, clinically applicable models (48).

The integration of digital health tools, computing power, and artificial intelligence has transformed healthcare, enabling personalized approaches to disease diagnosis, treatment, and prognosis. Computational medicine offers the potential to improve healthcare efficiency, reduce costs, and support proactive, individualized care by analyzing the interactions between biological and socioeconomic factors. However, challenges remain, including managing complex multiscale models, integrating diverse datasets, and ensuring safe clinical implementation. Realizing these opportunities requires interdisciplinary collaboration across scientific and medical domains (49).

Infrastructure Needed

Computer-Aided Drug Discovery and Development (CADDD) integrate computational tools to enable automated in silico drug discovery, supporting simulation management, computation, and structural visualization for users with varying expertise. The effectiveness of this approach has been demonstrated through performance evaluation of five docking programs using an HIV-1 protease dataset (50).

Drug target identification and lead discovery rely heavily on mathematical modeling, computational methods, and systems biology tools, which reduce cost, time, and failure risk compared to traditional experimental approaches (51, 52). Mathematical models assist in accurate target identification and analysis of drug-induced system behavior, although further tool development is needed to better represent complex biological systems (51-55). Figure 3 shows the process of the use of a mathematical model in the drug design process.

Quantitative drug design commonly begins with non-compartmental analysis and pharmacokinetic and pharmacodynamic modeling. Pharmacokinetics describes the temporal movement of a compound through biological spaces, whereas pharmacodynamics connects drug exposure to functional biological responses, enabling assessment of target engagement and therapeutic effect (56).

Facilitating practice improvements and building the capabilities

Computational approaches are increasingly central to drug discovery, enabling efficient identification of binding sites and mechanistic insights through multiscale biomolecular simulations. Virtual screening allows rapid exploration of large chemical

libraries for potential leads, while machine learning enhances prediction accuracy and efficiency in the era of big data. Integrating these methods can accelerate drug development and aid in discovering therapies with novel mechanisms. Multiscale modeling is particularly valuable for studying drug effects on excitable systems, such as cardiac ion channels, where interactions span atomic to functional levels. Structural modeling of drug–channel interactions support rational design of selective, high-affinity compounds for cardiac and neurological targets (57).

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