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BYPASSING THE PRECLINICAL STUDIES

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ABSTRACT Drug discovery is an integrated approach involving various domains, in the main goal of to discover a molecule for the therapeutic purposes. The routine drug discovery process involves lead identification, target prediction, and activity screening by respective in vitro, in vivo techniques and measuring its safety and efficacy in human volunteers. In vitro studies give a basic preliminary therapeutic activity of the lead molecule then it has been evaluated for the same activity and safety in suitable animal model. Screening of intended therapeutic activity in animal model is termed as preclinical or nonclinical studies. If the preclinical study gives positive result related to absorption, distribution, metabolism, elimination, toxicity and efficacy, the lead molecule considered as investigational new drug and it is further tested on human volunteers from Phase 0 to Phase IV. The data given by preclinical studies might useful to the research hypothesis but in the most of the preclinical studies, drugs that seem to be safe and effective in animal models typically turn out to be harmful or ineffective in humans because animal models often do not completely represent human physiology. Hence the aim of this review article is giving typical methods for bypassing the preclinical studies to prevent the animal sufferings.

KEYWORDS : Preclinical, insilico, 3D cell culture, Phase 0

Drug discovery is the process through which potential new medicines are identified. It involves some scientific disciplines, including pharmacognostic, pharmaceutical chemistry and pharmacology. A drug may be isolated from natural sources or synthesized in laboratory. Further it needs to identify an essential disease target for a specific therapeutic effect; the lead compound identification uses chemical libraries to test for activity against the target protein and optimization of the lead candidate. With the help of High throughput screening (HTS), drug target can be identified from the more complex assay system such as a cell-based and enzyme based assay.[1] This type of knowledge has given rise, more recently, to early discovery paradigms using pharmacophores and molecular modelling to conduct virtual screens of compound databases.[2] High throughput and other compound screens are developed and run to identify molecules that interact with the drug target, chemistry programmes are run to improve the potency, selectivity and physiochemical properties of the molecule, and data continue to be developed to support the hypothesis that intervention at the drug target will have efficacy in the disease state. It is this series of activities that are the subject of intense activity within the pharmaceutical industry and increasingly within academia to identify candidate molecules for clinical development.[3]

Whereas, pre-clinical evaluation includes *in-vivo* and *in-vitro* studies for the determination of tolerability, safety and efficacy of the compound of interest. Then the compound is filed for Investigational New Drug (IND) to Food and Drug Administration (FDA) to start the clinical trials. Clinical studies comprise five phases of clinical trials. Phase 0 is termed as micro-dosing phase, where minimum dose of IND given to small number of subjects (10-20 volunteers). Phase I clinical trials are conducted for the evaluation of absorption, distribution, metabolism and excretion (ADME) parameters of IND on 20–100 healthy volunteers. Phase II clinical trials are conducted for the determination of dose of IND on 100–500 healthy volunteers for a few months to years. Phase III trials are conducted on 1000– 5000 subjects for the determination of safety and effectiveness of the IND. Once the safety and efficacy were proved, then the IND filed to FDA as New Drug Approval (NDA). Finally the drug will be approved for post marketing trial that is Phase 4. Drug discovery and the development process include the investment of major capital ranging from millions to billions of dollars per successful drug.

Preclinical trials or nonclinical trials are conducted on animal models to test a new drug substance, vaccines or medical devices for the first time determination of safety and efficacy. There are many different species utilised globally, but the most popular ones are non-human primates, birds, fish, rabbits, guinea pigs, hamsters, mice, fish, rats, and other animals (monkeys, and chimpanzees). Almost 115 million animals are reportedly employed in experimental research annually across the globe. Animals' suffering, mortality, and misery during scientific studies have long been a topic of controversy.[4]

A lot of promising medications have failed to be safe and effective in human clinical trials despite promising preclinical studies. Approximately 85% of late-stage clinical trials of candidate drugs fail because of drug safety problems or ineffectiveness, despite promising preclinical test results. However the main objective is to collect the data to submit to the FDA for IND filing. Animal research will not be entirely replaced by other methods anytime soon, but the potential for faster and ultimately less expensive commercialization of therapeutics, together with the reduction of animal use in the early phases of drug development, has helped to increase research funding for alternatives.[4]

Increasingly, toxicological testing in animals and preclinical animal studies in drug development have been questioned because of poor correlation with in-human results.[5] Experiments on animals are cruel, time-consuming, and generally inapplicable to humans, the world's most forward-thinking scientists are developing and using animal-free methods that are actually relevant to human health for studying diseases and testing products. These alternatives to animal testing include sophisticated tests using human cells and tissues (*in vitro* methods), advanced computer-modeling techniques (*in silico* methods), and clinical studies (microdosing).

1. 3D tissue culture (organs-on-a-chip)

In order to assess drug absorption, distribution, metabolism, and excretion, cardiac function, and immunological response, 3D cell culture systems are being designed to replicate physiological organs in the body. The best course of action is to incorporate these organ-specific 3D models into a network of interconnected micro physiological systems that will allow for improved assessment of drug safety, efficacy, and disease modelling as well as the impact of various drug administration routes on drug pharmacokinetics.

3D cultures create intricate structures on top of a thick layer of extracellular matrix (ECM). Spheroids (anchorage independent systems) or cell culture scaffolds can be used to encapsulate cells (anchorage dependent). A complex microenvironment for 3D cell culture can be created using a combination of microfluidic tools, micropatterned plates with ECM components, and cultures in which produced spheroids are implanted in ECM scaffolds.[6]

Organoids are self-organizing, self-renewing 3D cultures derived from primary tissue, embryonic stem cells, or induced pluripotent stem cells that function similarly to the tissue from which they are derived. They have the potential to provide near-physiological conditions, but still have limitations such as the absence of a native microenvironment and lack of interactions with immune cells. Many different normal tissue and disease models of the digestive tract, prostate, lung, kidney, and brain have been characterized as organoid cultures, and applications in drug development and precision medicine are developing.[7,8]

Cell-based drug discovery has traditionally focused on chemical screens in well-characterized cell monolayers, but in recent years, 3D cell culture systems that model in vivo microenvironmental aspects are becoming more prominent in drug discovery. These models can take into account that the response to a broad spectrum of drugs varies not only with a particular cell line or tumor type, but also with its surrounding stroma. Models focused on enhanced cell motility, induction of cell dormancy, promotion of cell differentiation in epithelial cells and neurons, the support of stem cell-like properties or a desired microenvironment like that of a metastatic niche, enable the possibility of more specifically targeting certain cell behavior. Additionally, combining 3D cell cultures with primary patient-derived tumor cells and molecular profiling data or the formation of 3D organoid banks of tumor cells may open the door for preclinical screening of a personalized panel of drug candidates to improve outcome and reduce side effects of cancer therapy.[6]

An ideal 3D culture model would mimic a microenvironment where cells can multiply, aggregate, and differentiate that is physiologically or pathophysiologically relevant to a certain tissue or disease. Such a model would incorporate interactions between cells and the extracellular matrix (ECM), tissue-specific stiffness, oxygen, nutrition, and metabolic waste gradients, as well as a combination of scaffolding cells with tissue-specific properties.[9]

Three-dimensional (3D) cell culture systems have seen several advances, ranging from straightforward spheroids to more complex organs-on-chips, from static single-cell 3D models to 3D models for cell co-cultures with microfluidic flow control. Each has unique benefits and restrictions.

Microfluidic devices lined with living human cells for drug development, disease modeling, and personalized medicine. To mimic human organs in vitro, enabling faster, better, and cheaper drug development and insights into human health. These microdevices are composed of a clear flexible polymer about the size of a USB memory stick that contains hollow microfluidic channels lined with living human organ cells and human blood vessel cells. These living, three-dimensional cross-sections of human organs provide a window into their inner workings and the effects that drugs can have on them, without involving humans or animals.

Animal models often do not accurately reflect human physiology, meaning that drugs that appear to be safe and effective in animals frequently turn out to be harmful or ineffective in humans. This mismatch in biology causes many useless or toxic drugs to advance through clinical trials at great expense, while potentially effective compounds never make it to market. A better way to model human biology and diseases *in vitro* is needed to accelerate the development of new drugs and personalized medicine.

Organ-Chips involves crucial research on a variety of human illnesses and potential treatments for them, including cystic fibrosis, COVID-19, influenza, malnutrition, and radiation exposure. Cantex Pharmaceuticals' Investigational New Drug (IND) application to the FDA to start Phase 2 clinical trials for a medication it licenced from Harvard University to treat COVID-19 contained information from preclinical investigations using the Human Alveolus Chip from the Wyss Institute. This accomplishment opens the door for increased usage of Organ Chips in the process of developing and approving medications.[10]

2. Computational (*in silico*) methods

The fields of biomedical science and its related fields are entering a new era in which computational techniques and technology are expected to be widely used to assist team-based research on the human body.[11] More recently, artificial intelligence technologies are also gaining prominence along the whole target discovery process. Deep and machine learning tools allow researchers to automate and extract more meaningful information from experimental outputs, in order to generate models and build complex networks by integrating different data sources. Ultimately, these technologies greatly speed up the extraction process of potential therapeutic targets. However, results should be further validated through the exploitation of *in-vitro* and *in-vivo* methodologies.[12] To find new medications, more advanced *in-silico* methods are being applied. For consistent results, the majority of *in-silico* techniques are used in conjunction with *in-vitro* and *in-vivo* data.[13]

These computational techniques play a significant role in the pharmacology sectors that cover worldwide drug development through the use of various software and tools. Web servers for target prediction such as Open Targets Platform, TargetHunter, Poly pharmacology browser, SwissTargetPrediction, and MolTarPred, for ligand target prediction databases such as DrugBank, ChEMBL, and ChemBank, tools for QSAR modeling like QSAR-Co, Open3DQSAR, and QSAR ToolBox and for molecular docking analysis tools like GOLD, Glide, DOCK, AutoDock Vina and Discovery Studio were extensively used in the preliminary stage of the drug discovery process.[14]

The In Silico Oncology and In Silico Medicine Group[15] intends to produce multiscale simulation models of malignant cancer that are clinically driven and directed to be used as patient-individualized decision support and treatment planning systems after clinical adaption and validation.[16] They have led the development of the first technologically integrated Oncosimulator, a joint Euro-Japanese research venture.[17]

2.1. Virtual Physiological Human

Virtual Physiological Human (VPH) is centred on a methodological and technological framework that, when it is created, will allow collaborative research on the human body as a single complex system.[18] The collective framework will allow institutions and organisations to share information and findings, resulting in the development of diverse but interconnected computer models of the mechanical, physical, and biochemical operations of a living human body. A descriptive, integrative, and predictive framework is the VPH.[19-21] The framework should be descriptive, according to Clapworthy et al., by enabling laboratory and medical observations from all over the world "to be collected, categorised, structured, shared, and merged in any imaginable way." [21] It ought to be integrative by allowing related professionals to analyse those observations in a group setting in order to develop "systemic theories." Last but not least, it should be predictive by fostering links between extensible and scalable predictive models and "systemic networks that solidify those systemic hypotheses" while enabling observational comparison.[21]

Large digital collections of anatomical, physiological, and pathological data, typically predictive simulations derived from these collections, and services designed to aid researchers in the development and upkeep of these models as well as end-user technologies for use in clinical practise make up the framework. The goal of VPH models is to combine physiological activities on various time and length scales (multi-scale modelling).[22]

Combining patient-specific data with population-based representations is made possible by these models. Anatomical subsystems, dimensional scales (body, organ, tissue, cells, molecules), scientific fields (biology, physiology, biophysics, biochemistry, molecular biology, bioengineering), and reductionist approaches are not intended to be used in the development of a systemic approach to biological systems (cardiovascular, musculoskeletal, gastrointestinal, etc.).[23]

The ImmunoGrid is a grid-based application of a virtual human immune system. It offers tools for applications in clinical immunology, the design of vaccines, and immunotherapies, and it can model immunological processes at natural scales. The created toolkit will be used in clinical applications to develop immunotherapies for chronic infections and cancer after being verified with experimental data. Because the immune system is intricate and combinatorial in nature, experimental methods are expensive, and human experimentation is subject to limitations, computational models are useful.[23]

3. Clinical research (Phase 0 – microdosing clinical trial)

The absence of a therapeutic goal in phase 0 trials sets them apart significantly from trials carried out in accordance with a conventional IND. Sub-therapeutic yet pharmacologically effective drug doses are administered to study participants, who may be either patients or healthy volunteers. The amount of time that participants are exposed to the agent is restricted, but dose escalation is permitted as long as the goal is not to establish a safety/toxicity profile. The FDA permits more constrained (single-dose or short-course) preclinical toxicology studies to be performed to establish margin of safety rather than dose-limiting toxicities because low doses and drug exposures do not foresee severe drug-related adverse outcomes.[24]

Additionally, full-scale, clinical good manufacturing practice-grade commercial manufacturing is not necessary prior to trial beginning due to the little amount of study drug required to conduct a phase 0 trial. Phase 0 trials can therefore be started far earlier in the clinical development of an agent than standard phase I studies, offering a valuable chance to examine pharmacokinetic and drug target effects in people much earlier. Such pilot trials with a limited number of patients can provide information that can help with clinical development decisions and improve the design of subsequent trials. Following trials, such as accelerated (i.e., limited dose level) phase I studies, phase I trials combining targeted agents with cytotoxic medicines, or phase I/II, will go more quickly as a result of the human pharma cokinetic (PK) and pharmacodynamics (PD) results. A conventional IND application must be submitted in order to proceed with clinical evaluation in all of these subsequent steps because to the small quantity of study drug required to conduct a phase 0 trial. The authors tested ABT-888, an inhibitor of the DNA repair enzyme poly-ADP ribose polymerase, in patients with advanced cancers in the first phase 0 clinical trial of a therapeutic drug in oncology. The increasing use of the exploratory IND in the pharmaceutical industry also demonstrates the document's potential benefit in accelerating the conventional drug development pathway. Early in the clinical development phase, when deciding which prospective drugs to prioritise for further study, it is particularly helpful.[25-28]

4. CONCLUSION

A major reason why medications that enter clinical trials fail to advance to the market is because of prohibitive side effects or toxicity, which is a goal in drug development to predict undesirable side effects of potential therapeutic agents. Between Phase I trials and early post-market withdrawals,[4] far after significant time and money have been invested on what would turn out to be a "failed" therapy, animal toxicity research fails to predict toxicity in over 50% of medications in the pipeline. A "fail early" strategy, or testing that anticipates possible expensive late "drop outs" during drug development, would not only lower costs but also free up development resources to be put towards drugs with a higher chance of passing clinical trials. There are various computational tools for new drug discovery that could be cost-effective and quicker to help solve some of these issues. Through the use of in-silico methods, the number of possible compounds that can be examined for drug development can be reduced from the hundreds of thousands to the tens of thousands, saving time, money, and human resources. Non-animal model alternative methods efforts to replace, reduce, and refine (“3Rs”) the use of animal models in preclinical studies.[29] The most promising drug molecule is first identified in the conventionally accepted drug discovery method, which is then optimised *in-vitro*, *in-vivo*, and in pre-clinical investigations to determine whether the compound has the potential to be developed as a drug molecule. Likewise alternatives to animal research, such as cell and tissue platforms, computational *in-silico* modeling, 3D tissue research have shown great promise in facilitating drug development while decreasing time and expense, they have yet to make significant inroads as replacements for preclinical animal testing.

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