

A Review On Alternatives Of Animal Models In Experimental Pharmacology

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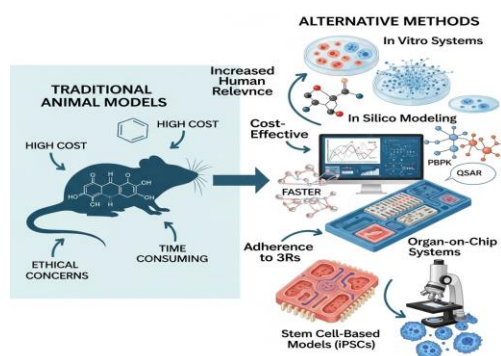
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ABSTRACT

In experimental pharmacology, animal models have long been essential for assessing the safety, effectiveness, and underlying mechanisms of action of drugs. Because of interspecies heterogeneity, using animals in study has created ethical issues and scientific constraints despite their historical significance. Experiments using animals are also frequently expensive and time consuming. These difficulties have prompted the creation and uptake of substitute techniques that can lessen or eliminate the need for animals in pharmaceutical testing. The utilization of in vitro systems, such as cell lines, primary cultures, and three dimensional (3D) organoids, is a significant substitute. These models provide regulated settings that make it easier to examine cellular reactions and allow for early toxicity and effectiveness screening. Another promising strategy is in silico modeling, which simulates drug interactions and makes predictions without using animal testing by using computational tools such as Physiologically Based Pharmacokinetic (PBPK) models and Quantitative Structure Activity Relationship (QSAR) models. Drug reactions can be more accurately analysed physiologically thanks to emerging technologies like organ-on-chip systems, which replicate the dynamic activities of human organs. Furthermore, stem cell-based models especially those made from induced pluripotent stem cells (iPSCs) allow for individualized evaluations and are useful for research on developmental toxicity. The human relevance, cost effectiveness, and general efficacy of medication research could all be greatly enhanced by these substitutes, which also adhere to the moral precepts of the 3Rs i.e., replacement, reduction, and refinement.

Keywords: Interspecies heterogeneity, three dimensional (3D) organoids, Physiologically Based Pharmacokinetic (PBPK), Quantitative Structure Activity Relationship (QSAR) models, induced pluripotent stem cells, replacement, reduction, refinement.

Graphical Abstract:



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1. INTRODUCTION:

In experimental pharmacology, animal models have long been essential instruments for drug discovery, toxicity evaluations, and the investigation of disease causes. Even if animals are useful, their continuous employment in research is coming under more criticism because of moral conundrums, high expenses, interspecies differences, and changing legal requirements. The scientific community has been prompted by these difficulties to investigate and use substitute techniques that seek to lessen, improve, or eliminate animal testing. The development of creative substitutes for animal models has advanced remarkably in recent years. Researchers can study medication effects in controlled cellular environments using in vitro techniques like two-dimensional (2D) cell cultures, primary cells, and three-dimensional (3D) organoids. These techniques provide important information about tissue-specific responses. Similarly, without the use of live subjects, in silico methods specifically, computational tools such as physiologically based pharmacokinetic (PBPK) models and quantitative structure activity relationships (QSAR) can forecast the pharmacological and toxicological behaviour of compounds through mathematical simulations. [1]

In recent years, there has been a notable advancement in the creation of innovative alternatives to animal models. Using in vitro methods such as two-dimensional (2D) cell cultures, primary cells, and three dimensional (3D) organoids, researchers can examine the effects of medications in controlled biological environments. Important details regarding tissue-specific reactions are provided by these methods (Hartung, 2009). Similarly, in silico approaches that is, computational tools like quantitative structure-activity relationships (QSAR) and physiologically based pharmacokinetic (PBPK) models can predict the pharmacological and toxicological behavior of compounds through mathematical simulations without the need of live.

The development of organ-on-chip and microfluidic systems, which replicate the physiological processes of human organs on a microscale, is another exciting development. These systems offer dynamic environments for observing drug interactions across organ systems, in addition to increasing the relevance of preclinical testing. All things considered, these substitutes offer better efficiency and translational accuracy in drug development while also abiding by the moral precepts of the 3Rs Replacement, Reduction, and Refinement. These technologies are fast evolving and make a substantial contribution to modern pharmacological research, even though the complete replacement of animal models is still a goal for the future. [2]

1.1 Three R's: Reduction, Refinement and Replacement

The Three R's i.e., Reduction, Refinement, and Replacement are the guiding principles for the moral use of animals in experimental pharmacology. These guidelines seek to preserve scientific integrity while ensuring that animals are treated humanely. Reduction is the process of using fewer animals in research without sacrificing the accuracy of the results⁹. This is accomplished by using sophisticated statistical techniques, meticulous experimental planning, and encouraging data sharing to prevent needless duplication. Reliance on animal testing is further lessened by methods like computer-based models and high-throughput screening. The ethical use of animals in experimental pharmacology is guided by the Three Rs: Reduction, Refinement, and Replacement. These rules aim to protect the integrity of science while guaranteeing the ethical treatment of animals. Reduction is the practice of employing fewer animals in studies without compromising the precision of the findings. Sophisticated statistical methods, careful experimental design, and promoting data sharing to avoid unnecessary repetition are used. Techniques like high-throughput screening and computer-based models help reduce the need for animal experimentation even more.

1.1.1 Reduction

The goal of reduction is to utilize as few animals as possible in research while still producing data that are reliable from a scientific standpoint. Carefully thought-out study designs and statistical optimization can help achieve this. In vitro cell culture methods, for example, are useful for early chemical screening, assisting in the identification of harmful or inefficient drugs prior to animal testing. Early in the drug development process, researchers can identify inappropriate substances by using human hepatocyte cultures to evaluate how a drug is likely to be metabolized and eliminated.

Similarly, in vitro embryonic stem cell culture testing can improve embryo toxicity investigations that have historically relied on live animals and embryos. These models greatly reduce the number of live embryos required by predicting the embryotoxic potential of chemicals.

Furthermore, by avoiding repeated animal tests, data sharing such as previously collected information regarding excipients and medicine formulations contributes even more to reduction. By minimizing the needless use of animal models, these strategies together guarantee moral, effective, and scientifically sound research procedures. [3]

1.1.2 Refinement

The goal of refinement is to reduce the amount of pain, suffering, and discomfort that lab animals endure both during their lives and during experiments. Improving the atmosphere in animal housing is a crucial tactic that promotes natural behaviour's and lessens stress. For instance, it has been demonstrated that allowing animals to nest, hide, gnaw, and forage in enriching cage habitats considerably lowers stress levels. Hormonal imbalances brought on by stress and discomfort in animals may result in inconsistent experimental outcomes. Consequently, this frequently calls for repeating research, which raises the quantity of animals employed. Refinement increases scientific data's dependability and repeatability while also improving animal welfare. Research using genetically altered mice to simulate Huntington's illness provides a noteworthy example. Compared to mice confined in barren cages, these mice showed slower disease progression and more closely resembled human disease patterns when housed in enriched surroundings. These improvements produce better experimental models and more humane circumstances, which promotes scientific progress and ethical responsibility. [4]

1.1.3 Replacement

For scientific investigation and testing, replacement refers to using lesser creatures or non- animal substitutes in place of higher animals. In vitro models, cell cultures, computer simulations, and sophisticated imaging and analytical tools are some of the alternative approaches that have been developed. By observing cellular responses in controlled settings, in vitro systems give researchers important early insights that can forecast results in vivo. For instance, instead of using live animals for testing, computer models have been used to mimic heart function and find interesting treatment candidates. The number of animals employed in cosmetic and pharmaceutical safety studies has drastically decreased in many countries as a result of the replacement of old animal-based assays like the Draize eye and skin irritancy tests with in vitro cell culture techniques. The synthesis of insulin, which was previously isolated from the pancreases of cows and pigs, serves as another illustration. Insulin is being produced utilizing recombinant bacterial cultures, which increases its accessibility and morality for diabetics. Although testing the purity and effectiveness of insulin was previously frequently done on animals, these tasks are now carried out using contemporary methods like chromatography.

Together, these developments show how replacement techniques can significantly cut down on or do away with the requirement for animal testing while preserving scientific integrity and enhancing applicability to human health. [5]

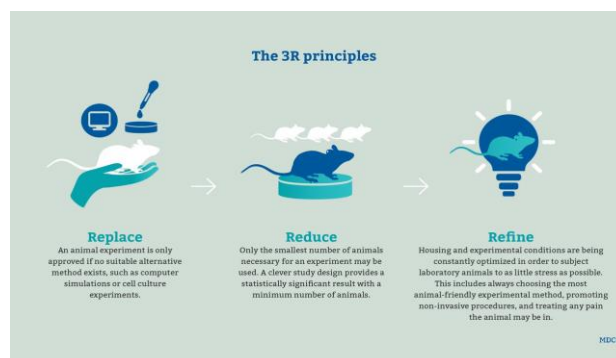


Fig. 1. The 3R Principles

2. ALTERNATIVE METHODS OF ANIMAL MODELS

Numerous creative strategies have been put forth to lessen or do away with the use of animals in experimental research. At least in some phases of research, these alternate techniques provide efficient alternatives for evaluating medications and chemicals. Reduced expenses, less staff needed, and more time efficiency are some of their main advantages. These alternative methods and their uses in toxicological and pharmacological testing are explained in full below.

2.1 *In Vitro* Models

Under carefully monitored laboratory circumstances, *in vitro* models such as cell lines, primary cell cultures, and three-dimensional organoids are frequently utilized to investigate pharmacological effects at the cellular and tissue levels. High-throughput screening is made possible by these models, which also lessen the need for animal experimentation. For instance, the use of human hepatocyte cultures to evaluate drug metabolism and forecast liver toxicity is a noteworthy, offering preliminary information on the safety and effectiveness profile of a molecule. These models are becoming more useful in preclinical research to find potentially hazardous substances before moving on to *in vivo* investigations. [6]

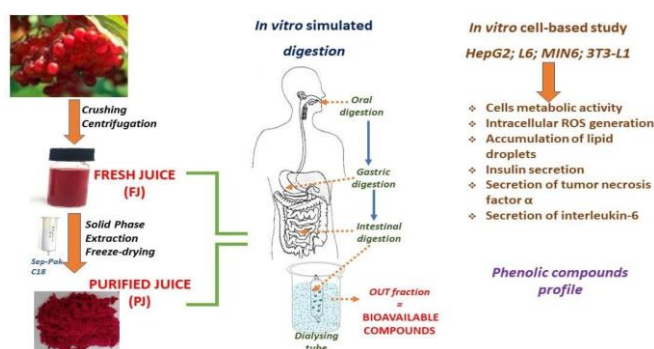


Fig.2. *In vitro* digestion process

2.2 *In Silico* Models

A non-invasive, economical, and morally sound method of comprehending the pharmacokinetics (PK), pharmacodynamics (PD), and toxicity of possible drug candidates is provided by *in silico* models, which are strong instruments in pharmaceutical research. These models do away with the necessity for substantial early-stage animal testing by forecasting how a medicine would behave in the human body using computer-based algorithms and simulations. Physiologically Based Pharmacokinetic (PBPK) modelling and Quantitative Structure-Activity Relationship (QSAR) models are two prominent *in silico* methodologies.

By using virtual populations, PBPK modelling enables researchers to predict the Absorption, Distribution, Metabolism, and Excretion (ADME) of medications across a range of organs and tissues. In order to improve dosing schedules and lower the possibility of side effects, it offers comprehensive forecasts regarding medication concentration throughout time. On the other and, QSAR models allow for the quick screening of huge chemical libraries to find compounds with desired qualities by correlating chemical structures with biological activity or toxicity. The drug development pipeline's efficiency is greatly increased by these *in silico* methods. They save time and money by identifying hazardous or ineffective substances prior to clinical trials, which aids in early-stage decision-making. Furthermore, by reducing the dependence on animal models, *in silico* techniques meet ethical norms in scientific research and are consistent with the 3Rs (Replacement, Reduction, and Refinement) approach. Apart from drug development, *in silico* methods are useful for environmental risk assessments, regulatory filings, and the creation of tailored medicine plans. The future of pharmaceutical research is anticipated to be significantly shaped by *in silico* modelling as biological datasets and processing power continue to grow. [7]

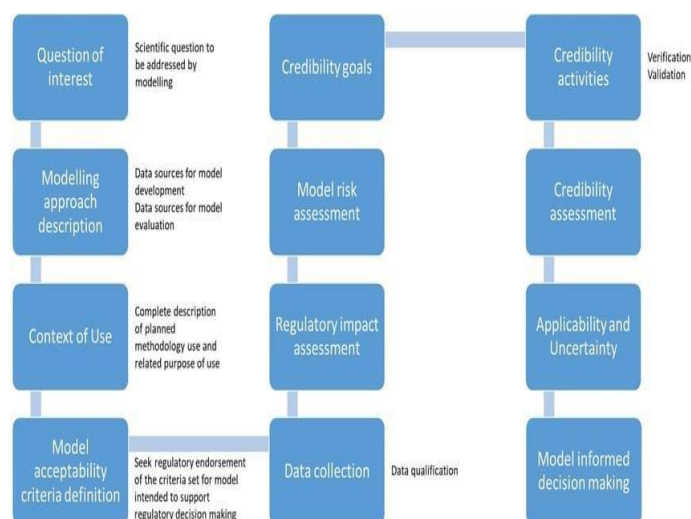


Fig. 3. Detailed Process of In-silico Model

2.3. Organ-on-Chip Technology

Organ-on-a-chip technologies, also known as microfluidic devices, are becoming increasingly sophisticated instruments in drug development and biomedical research. To simulate the dynamic physiological milieu of organs like the liver, lungs, heart, or kidneys, these devices use living cells inside carefully designed channels to recreate the structure and functionality of human organs on a tiny scale. Liver-on-a-chip and lung-on-a-chip systems are two examples; they are made to mimic organ-specific processes including gas exchange and metabolism. These technologies' capacity to enable real-time monitoring of drug effects, such as toxicity, effectiveness, and metabolism, in circumstances that closely mimic those seen in the human body is one of their main advantages. Compared to conventional in vitro or animal models, this leads to more accurate predictions of human reactions. Furthermore, by making it possible to test on patient-specific cells, microfluidic technologies aid personalized treatment. All things considered, these developments have the potential to minimize animal testing, lower research costs, and increase drug safety. [8]

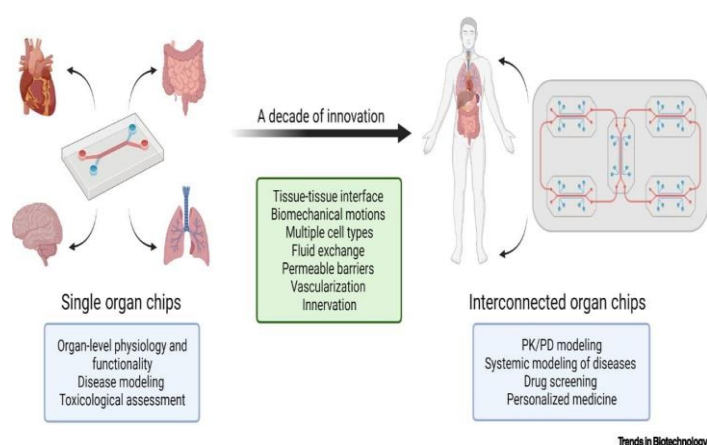


Fig. 4. Process of Organ-on-chip Technology

2.4 Stem Cell-Based Models

Embryonic stem cells and induced pluripotent stem cells (iPSCs) are potent substitutes for conventional animal models in drug development and scientific research. Because of these stem cells' exceptional capacity to develop

into multiple human cell types, highly relevant tissue and organ models can be produced in vitro. In a controlled laboratory setting, this capability enables researchers to explore disease causes, evaluate drug reactions, and model complicated organ systems. iPSCs obtained from individual patients are particularly useful in the domains of precision therapeutics and personalized medicine since they may be used to predict patient- specific medication responses and model hereditary disorders. Additionally, stem cell-based models are crucial for more efficiently screening possible medication candidates and assessing developmental toxicity. These models enhance the translational accuracy of preclinical research by precisely simulating human physiological and pathological processes. Stem cell technologies not only improve research ethics but also lead to more dependable and human-relevant scientific results by eliminating or minimizing the necessity for animal experimentation. [9]

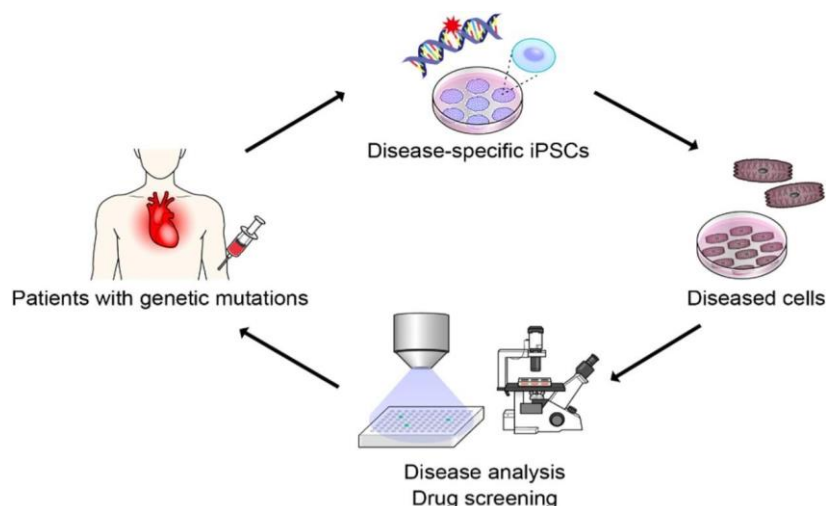


Fig. 5. Induced pluripotent stem cells -based drug screening

Table1: Summary of Alternatives of animal models in experimental pharmacology

S.NO	MODEL	DESCRIPTION	MECHANISM
1	In Vitro Models	Experiment using isolated cells or tissues in a controlled environment	Human or animal cells are cultured and exposed to drugs responses like toxicity metabolism and efficacy are measured.
2	In Silico Models	Computer stimulations and modelling	Uses algorithms to predict drug behaviour. [Ex: ADMET properties target binding] based on chemicals.
3	Organ-on-Chip Technology	Microfluidic devices that mimic the microarchitecture of organs	Stimulates blood flow, mechanical forces and multiorgan interactions; allows real time monitoring of drug effect.

4	Stem Cell-Based Model	Use of human or animal cell lines	Cells [ex : hepatocytes for liver studies] are used to evaluate drug absorption, metabolism and toxicity.
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3. FUTURE SCOPE:

3.1 STRATEGIC MOTIVATORS

- Human Relevance & 3Rs: Replace, reduce, and improve the usage of animals; switch to human-predictive models.
- Regulatory Opportunities: Using NAMs and MPS when making decisions (e.g., OECD guidelines, U.S. 2022 policy).
- Cost & Throughput: Prior to sophisticated testing, drug screening is quicker and less economical.
- Mechanistic Insight: Use of omics, live imaging, and CRISPR for deeper biological understanding. [10]

3.2 NEW PLATFORMS WITH INCREASING FUNCTIONS

3.2.1. Tiny, ethically Acceptable Organisms

- Zebrafish: large-scale imaging, heart, brain, and developmental safety; whole- organism pharmacology.
- Fruit flies are models for neurodegeneration, metabolism, heart problems, and epilepsy. They have fast genetics.
- Elegans: Behavioural tests such as chemotaxis; aging, neurotoxicity, and proteostasis. Research on infection, antimicrobials, angiogenesis, and oncology; link to mammalian models Galleria mellonella & CAM12. [11]

3.2.2. Systems Reconstructed by Humans

- Organoids: Immune/endothelium co-culture; illness and toxicity tests particular to a patient. [12]
- Organs-on-Chips: Microfluidic tissue models for toxicity and PK/PD in the liver, kidney, lung, blood-brain barrier, and heart. Preserve tissue architecture for studies on fibrosis and metabolism with 3D bioprinted tissues and precisely cut slices. [13]

3.2.3. Hybrid & In Silico Methods

- Forecast human exposure, variability, and dosage methods using the PBPK and QSP models. [14, 15]
- AI/ML Screening: Integrated phenomics from model organisms and forecast ADME/Tox and off-targets. [16]

4. FUTURE EXTENT

Standardization includes replication, reference compounds, and standardized procedures.

Human Diversity: iPSC panels that represent socioeconomic and genetic diversity. [17]

Omics-First Readouts: Transcriptomic/epigenomic trends for early toxicity detection. [18]

Digital Twins: Customized QSP models that are updated in real time using laboratory data. [19]

Ethics-by-Design: Data equity and the management of materials derived from consumers. [20]]

4.1 Model Advantages & Ideal Applications

Zebrafish, Drosophila, and C. elegans are used for early screening in order for carrying out quick phenotypic assays.

- Mechanistic Safety: Multi-omics organoids and organs-on-chips. Galleria mellonella and CAM for revascularization and antimicrobial research in infectious disease and oncology. [21]
- PK/PD & DDI: PBPK techniques + MPS for investigating drug metabolism and interactions.

iPSC organoids coupled with QSP for patient-specific treatment planning is precision medicine. [22]

4.2. Models in Clinical Translation Examples

A. MINIATURIZED MODEL ORGANISMS

Zebrafish: Used to detect cardiovascular adverse effects of QT-prolonging medications; results confirmed by ECG tests on humans. Kinase inhibitors for neurodegenerative illnesses were discovered in Drosophila and are now undergoing clinical trials.

Neuroprotective substances that passed this screening in C. elegans advanced to early-stage clinical trials. [23]

B. SYSTEMS DERIVED FROM HUMANS

Patient-Derived Organoids: These have a high degree of agreement with real clinical reactions and are used in oncology to predict patient-specific medication responses. Organs-on-Chips are FDA-approved platforms for forecasting important clinical endpoints such as lung absorption, blood-brain barrier penetration, and liver toxicity in humans. Prior to human dermatology trials, 3D bioprinted human tissues were used in investigations on skin irritation and wound healing. [24]

C. HYBRID/COMPUTATIONAL MODELS

Models of PBPK Connected to MPS Data: Forecast drug interactions, clinical pharmacokinetics, and dosage for certain populations (e.g., paediatrics, renal impairment). [25]

5. ALTERNATIVE ANIMAL MODELS IN EXPERIMENTAL PHARMACOLOGY – DISCUSSION

• In pharmacology, the three Rs

Reduction: Data exchange, in vitro pre-screening, and biostatistics lead to fewer lab animals.

Refinement: Use analgesics, compassionate objectives, and optimal housing to reduce pain and stress.

Use recombinant systems, in vitro (cell/organoid), and in silico (PBPK/QSAR) in place of animals. [26]

• Important Platforms

Cell lines, organoids, early safety, and metabolism testing are all done in vitro. PBPK, QSAR → forecast ADME/Tox, improve dosage in silico.

Organ-on-Chip: Real-time efficacy and toxicity based on simulated organ activity. iPSC/ESC Stem Cell Models: Drug Response and Disease Specificity by Patient. [27]

• Drivers

Ethical compliance and human prediction (3Rs). Regulatory assistance (FDA, OC D, NAMs). Quicker and less expensive early discovery. Deeper understanding of the mechanism (live imaging, CRISPR, and genomics). [28]

• Examples of Clinical Translation

Zebrafish:

QT prolongation predicts cardiotoxicity. Trials on Drosophila: Neurodegeneration targets.

Neuroprotective in elegans: Preliminary clinical trials. Organoids: Oncology medication response prediction. FDA-qualified toxicity prediction for organs-on-chips. [29]

- **Future Scope**

PK/PD using a multi-organ "body-on-chip." Standardized datasets and protocols. For genetic diversity, use iPSC panels. For accurate dosage use digital twins. Design-based ethics for human resources. [30]

CONCLUSION

Animal models used in experiments have long been vital resources for researching human biology and disease processes. However, there is an increasing trend toward alternate research methodologies due to growing ethical concerns and legislative limits regarding animal welfare. These substitutes, which offer more ethical, cost-effective, and human-relevant methods for drug testing and biomedical research, include in vitro digestion models, stem cell- based systems, organ-on-a-chip technologies, and in silico computational models (Hartung, 2009; Bhatia & Ingber, 2014). This review emphasizes these cutting-edge methods as potential substitutes or enhancements to conventional animal testing in toxicology and pharmacology.

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Conflicts of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Ethics approval and consent to participate

Not applicable. This review article does not involve any studies with human participants or animals performed by any of the authors.

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