Animal Models for Cancer Research: The Choice of the Right Model System

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Sinan Kandir

Contents

Abstract

The human is a complex organism, so if translational research is conducted, it should similarly mimic that complexity. Model systems comprise mathematical, computational, in silico, ex vivo, in vitro, and in vivo models in cancer research. Alternative model selections are the best practice for the reduction of experimental animal usage. The aim of animal usage in cancer research is to well-understand the physiopathology of different types of cancer, from genomics/proteomics to metabolomics levels, to screen the behaviors of the cancer cells in living organisms, and the efficiency of the treatment methods that mirror precision medical areas. Various animals can be used as model organisms. The most important point in experimental animal usage is ethics. This chapter will primarily focus on the fundamentals of the model systems with the comparisons of in silico and in vitro as alternatives to animal models. Then, the chapter will discuss the in vivo models

S. Kandir
Department of Physiology, Faculty of Veterinary Medicine, Cukurova University, Ceyhan, Adana, Turkey
e-mail: sinankandir@cu.edu.tr

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with ethical issues of animal experimentation, the R principles, and the selection of the suitable animal models in cancer research.

Keywords

Animal · Cancer · Humanized · Oncology · In vivo · 3R

Introduction

The high-throughput, accurate, robust, validatable, reproducible, and transparent data are vital in order to maintain high-quality research and obtain translatable results. The primary step of a state-of-the-art study begins with planning from head to toe and a well-designed methodology, while model selection is the core step. The animal models are essentially used for biomedical research to understand physiological and physiopathological conditions, and to develop new therapeutics for centuries. The most important point in experimental animal usage is ethics. Animals are living organisms like humans, and modern humankind could occasionally neglect its position through the evolutionary axis. Thus, acquiring knowledge about legal regulations and ethical rules, in particular the 3Rs, is the basic procedure of biomedical research before taking action. Furthermore, alternative model selections are the best practice for the reduction of experimental animal usage.

Alternative Model Systems

The model systems in biomedical research could be defined as controlled experimental setups that are mimicked similarly or identically target organisms (human or animal) systematically with reproducible, inspectable, and transparent features for developing hypotheses to understand the mechanisms and discover solutions to complex biological problems.

In Silico Model Systems

Mathematical oncology is modelling and simulating cancer models by using applied mathematics with knowledge of calculus, differential equations, statistics, and mathematical theories (e.g., game theory, Heisenberg's uncertainty principle, chaos theory, fractals, quantum mechanics, etc.), to predict the cancer dynamics and behavior, personalized medicine, and effectiveness of treatments (Anderson and Maini 2018; Anderson and Quaranta 2008). Because of reducing the number of experimental animals and robust reproducibility, mathematical oncology has gained importance exponentially (Anderson and Maini 2018). The road map of mathematical oncology has not only focused on cancer dynamics, therapeutic response, and personalized medicine, but also evaluated patient-specific big data and improve early

detection strategies with the statistical science in the last decade (Rockne et al. 2019; Anderson and Quaranta 2008). These types of models are named *mechanistic models* which are integrated whole data incomes from patients or experiments to clinical outcomes (Baker et al. 2018; Gaw et al. 2019).

The primary motivation of mathematical oncology is the transference of big data to clinical predictions of the likelihood of real scenarios (Rockne and Scott 2019; Rockne et al. 2019). The mechanistic models in cancer research are integrated with the mathematical formulas into machine learning and test the prognostic hypotheses and predictions with the offer of the best treatment options. Hence, basic cancer research takes advantage of *intersection shapes richness* – an ecological concept that defines the richness of biodiversity at the intersection areas – of various disciplines, from mathematics, physics, and biology to computational science. However, mathematical models can mostly focus on a specific and small area in the face of cancer complexity. The recent advances in computer science have boosted the mathematical model systems to a more reliable form of computational biology. The computational models which originate from mathematical formulas are becoming backbones in cancer research (Gaw et al. 2019; Anderson and Quaranta 2008; Anderson and Maini 2018; Baker et al. 2018; Bekisz and Geris 2020).

In this perspective, the in silico models are established. Basically, the frameworks of in silico models are the way of the translational phase of fundamental mathematical formulas to computer programs, bioinformatics knowledge with machine learning and artificial intelligence, to –omics area, and straightforward to silicon chip technologies of microfluidic physiological systems (microphysiological systems; lab-on-a-chip, organ-on-a-chip) to clinical applications. These sophisticated, cost-saving, flexible, lab-handled in silico model systems have promise for the future owing to a great opportunity in the preclinical cancer research with virtual screening of the cancer dynamics, drug design to interactions, treatment efficiency in nano-scale, and also, quite favorable in respect of R principles (Stillman et al. 2020; Niarakis and Helikar 2021; Jean-Quartier et al. 2018). In spite of the offered advantages of in silico model systems, these are still juvenile in comparison to in vivo models, and have pitfalls and limitations such as inability to simulate whole organisms throughout cancer homeostasis and allostatic mechanisms, algorithmic challenges and complexity, and need large-scale datasets to produce accurate computational data (Fig. 1) (Sacan et al. 2012; Bray 2015).

In Vitro Model Systems

The in vitro model systems are powerful candidates of alternative models for animal experiments because of their adjustable and easy integration capabilities with in silico models. Literally, knowledge about the history of the in vitro models started with microbiological and pharmacokinetic studies in the early 1950s (Grasso 1985). The cell-based in vitro models are preferred owing to their cost-effective and time-shortened nature, well-controlled environmental circumstances, enabling them to study specific cell lines with distinctive molecular pathways, ethical flexibility,

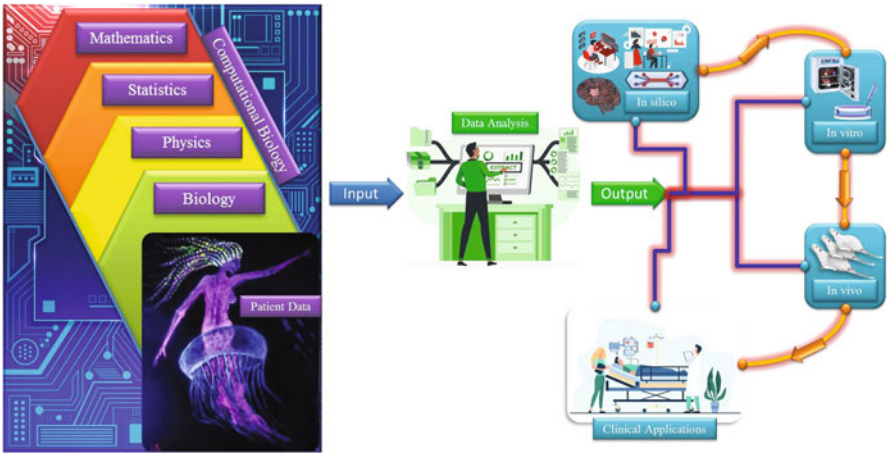

Fig. 1 Cancer research models, from basic science to clinical applications

standardization, and reproducible properties (Arantes-Rodrigues et al. 2013; Nikolic et al. 2018; Katt et al. 2016). Current scientific biotechnological innovations have paved the way for ultrafast developments in cutting-edge in vitro model technologies. Classical in vitro models have been comprised of two-dimensional (2D) monolayer cell cultures coated on a plate by a selected cell line; they are inaccurate to mimic a dynamic tumor microenvironment (complex cellular and extracellular matrix interactions). Thus, recently, more complex spheroids and organoid (organ-like) models via 3D cell cultures have been produced that enable mechanically active and reliable simultaneous molecular response (Yip and Cho 2013; Birgersdotter et al. 2005; Rodrigues et al. 2021). Furthermore, 4D semi-active organoids are available, which are more similar to the real tissue with extracellular matrix, heterogeneity, vascularization, epithelial tissue properties, regulable and dynamic microenvironment, such as ex vivo models besides 3D matrix composite cell lines (Fig. 2) (Jensen and Teng 2020; Charbe et al. 2017; Zhao et al. 2021; Langhans 2018; Wessels et al. 2022; Kuhl et al. 2016). *Tech-feed-tech*, so, contemporary 3D bioprinting technologies shed light on rapid improvements to in vitro model systems. The development of in vitro models facilitates the translational potency of basic science to clinical applications.

The 2D models are limited, due to their monolayer single-cell designed homogeneous structure, by cellular drug response and screening the basic cellular behaviors (migration, proliferation, apoptosis, etc.), whereas the 3D models are used to mimic the tumor microenvironment, metastatic behaviors; moreover, the 4D models are more realistic and complex than other in vitro models and comprise high-throughput imaging analysis by adding in silico data, with their heterogeneous microenvironment properties, progressive, metastatic, individual, and collective behaviors of the cancer cells, therapy response, and resistance (Wessels et al. 2022; Kuhl et al. 2016; Rodrigues et al. 2021; Zhao et al. 2021; Yip and Cho

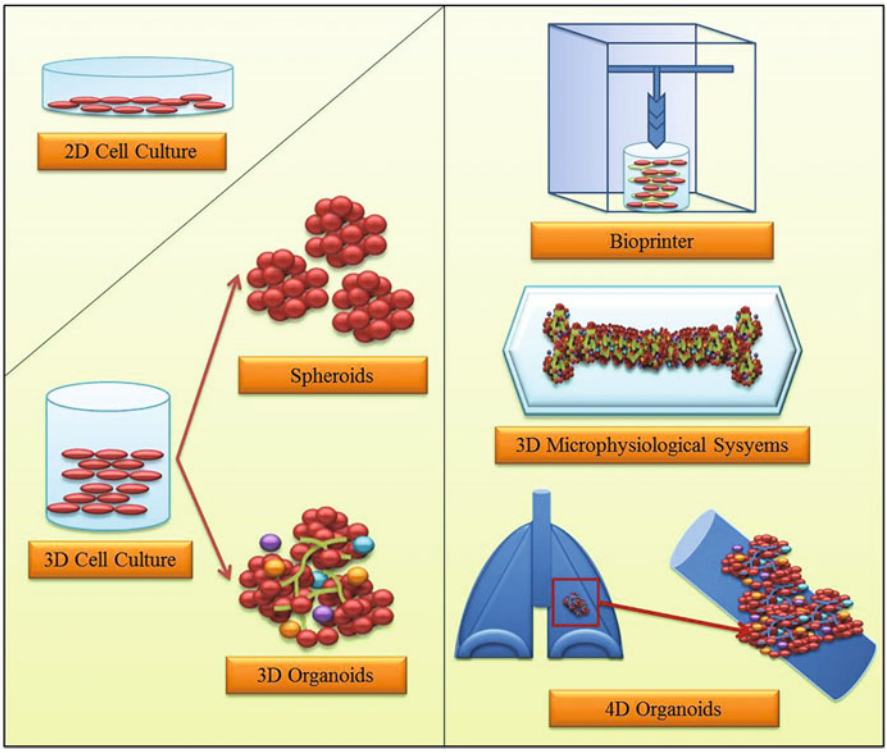

Fig. 2 In vitro cell culture models

2013; Gao et al. 2016). Nevertheless, high-tech 3D and 4D in vitro models still have some disadvantages in comparison to 2D, including methodological difficulties, more expensive infrastructures, time-consuming, and low reproducibility (Bartlett et al. 2014; Kapalczynska et al. 2018; Wan et al. 2020; Tibbits 2014; Gao et al. 2016).

In Vivo Model Systems

Cancer growth and metastasis are dynamic processes, and inside the living organism, numerous factors get involved, including intermediary metabolism and homeostatic and allostatic mechanisms. Animals have been used as experimental models in cancer studies to reveal the tumorigenic mechanisms and treatment options for over more than a century. Subsequent to Rudolf Virchow's chronic irritation and Julius Cohnheim's embryonic rest hypothesis, Johannes Fibiger succeeded in inducing papilloma and carcinoma in wild type piebald rats' esophagus and stomach by *Spiroptera neoplastica* – currently known as *Gongylonema neoplasticum* – in 1907 and was awarded Nobel Physiology and Medicine in 1927 (Nobel 1927). Then,

Yamagiwa and Ichikawa (1977) induced epithelial carcinoma by chronic irritation with coal tar painting for the first time in the laboratory rabbits. Since the discovery of the Rous sarcoma virus (described as an oncogene) in 1910 by Peyton Rous (Nobel Prize in 1966), who identified the cause of malignant chicken sarcoma and triggered new spontaneous cancer models in hens with allogeneic transplantation, research has been carried out on new animal cancer models. Thereafter, Harold Varmus, J. Michael Bishop (Nobel Prize in 1989), Dominique Stehelin, and Peter Vogt found the cellular origin of retroviral oncogenes of avian sarcoma virus, which leads to the new paths for identification of human oncogenes (2021). In spite of the rapid developments on in silico and in vitro models, the in vivo model systems are still essential for translational research including preclinical studies in cancer.

Types of Animal Models

Principally, the animal models could be divided as small and large animals (Ziegler et al. 2016; Mondal et al. 2022; Kandir 2021). Small animals are mostly preferred by researchers due to their well-controlled, easy-handled, cost-efficient, standardized with have a short reproductive cycle and life span advantages. On the other hand, large animals are useful models not only for anatomical or physiological similarities with humans, but also have spontaneous cancer types such as lymphomas, adenocarcinomas, mammary tumors, skin, pancreas, colon, bladder, and prostate cancers, etc. (Pinho et al. 2012; Ziegler et al. 2016; Giuliano 2021; Biller et al. 2016; Hudachek et al. 2010; Schmahl et al. 1978). While the rabbits were selected as experimental model animals initially, the rodent models (mice and rats) are the most preferred animals currently because of their inbred, homogenous and standardized colonies with detailed knowledge about their genetic backgrounds (Mouse Genome Sequencing et al. 2002; Gibbs et al. 2004). As shown in Table 1, the researchers have miscellaneous alternative animal sources and models in order to establish their cancer studies.

Although mouse and rat models are the most preferred animals by cancer researchers due to their highly standardized inbred strains, the large animal models especially pigs and dogs have more similarities to humans with their genetic heterogeneity. Whole listed animals in Table 1 have own genome projects to screen high-throughput sequenced genomes with single nucleotide polymorphism (SNP) datasets (Denoyelle et al. 2021; O'Brien et al. 2002; Ostrander and Kruglyak 2000; Archibald et al. 2010; Alföldi et al. 2009; Howe et al. 2013; Keane et al. 2011; Mouse Genome Sequencing et al. 2002; Wade et al. 2009; Chimpanzee and Analysis 2005; Zorio et al. 2019; Bovine Genome et al. 2009; Romanenko et al. 2015; Adams et al. 2000; Gibbs et al. 2004). This knowledge gathers many advantages for cancer researchers to design their studies. The researchers have to ask the right questions to themselves for the animal experimentation, as "*How will I set up my animal experiment design?*" and "*Would this experiment be translational to humans?*". The aims of the researcher have to be realistic and result oriented for the ultimate patient. Hence, the well-designed, randomized, blinded, controlled experiments

Table 1 Animal models for cancer research (Kemp 2015; Peng et al. 1993; Giuliano 2021; Biller et al. 2016; Raby et al. 2020; Kamdem et al. 2020; Hudachek et al. 2010; Palmarini and Fan 2001; Schachtschneider et al. 2017; Tian et al. 2020; Hicks et al. 2021; Xia and Chen 2011; Schmahl et al. 1978)

Small animal models		Chemically induced	Spontaneous	Tumor transplantation	Genetically engineered	Humanized
	Mice	*	*	*	*	*
	Rat	*	*	*	*	*
	Rabbit	*	*	*	*	
	Gerbil	*	*	*	*	
	Guinea pig	*	*	*	*	
	Zebrafish	*	*	*	*	*
	Chicken	*	*			
	Drosophila	*		*	*	
Large animal models	Pig	*	*	*	*	*
	Dog	*	*			
	Cat	*	*			
	Cattle		*			
	Sheep		*			
	Goat		*			
	Horse	*	*			
	Nonhuman primates	*	*	*	*	

provide a powerful tool for gaining new knowledge. By this aim, chemically induced, spontaneous, syngeneic, xenograft, genetically engineered (GE), and nowadays, humanized animal models are used to establish the in vivo setup of cancer studies.

Chemical agents, toxic substances and their intermediate products are involved in every aspect of our lives. Environmental exposures to these agents induce carcinogenesis, especially in the epithelial tissue. Basically, chemical carcinogens are classified as genotoxic (polyaromatic hydrocarbons, alkylating agents, aromatic amines and amides, etc.), which are driven by DNA damage directly or indirectly, and non-genotoxic (cytotoxic, receptor-mediated, hormonal disruptors, oxidants), which act for prolonged periods with indirectly altered cellular homeostasis, hence leading to spontaneous tumors.

"*Why and when should researchers choose chemically induced models?*" To address this question: chemically induced models contribute to the characterization of toxic mutagens, to screen DNA damage and repair mechanisms, chemoprevention, or the early diagnostic approach. The mice and rats are major models for that type of research due to their well-known genetic backgrounds and ensured genetic homogeneity with inbred strains. Moreover, canine and feline models are suitable for chemical-induced carcinogenesis because of long time exposure to the same environmental pollutants and genetic heterogeneity similar to humans (Yuspa and Poirier 1988; Takashima-Uebelhoer et al. 2012; Hayes et al. 1991; Schmahl et al. 1978).

Each animal have their spontaneous cancer traits. The main question in spontaneous cancer research is that "*What is the similarity rate of cancer compared to humans?*". For example, urothelial carcinoma known as transitional cell carcinoma (TCC) occurs in both humans and dogs with the same origins such as chemical exposure to smoking, organochlorine pesticides, arsenic-contaminated or chlorination by-products of the water that are associated with polymorphisms on glutathione S-transferases (GSTs) genes (G > A in *GSTT1* or 6 bp deletion in *GSTT5* exon4) which eliminates GST enzyme activity (Luethcke et al. 2019; Craun et al. 2020). In this view, the researcher might use the animal-specific genome and SNPs databases by bioinformatics tools to match selected cancer types in animals versus humans.

Tumor transplantation is another option for understanding cancer cells' behavior, mechanism of tumorigenesis, metastatic and invasive features, and alternative therapeutics in preclinical research. Herewith, patient-derived tumors are transplanted either to selected athymic or genetically engineered severe combined immunodeficient (SCID), immunocompromised or pharmacologically immunosuppressed or immunocompetent humanized animals – to keep from graft versus host reaction – named as patient-derived xenograft models (PDX) by orthotropic implantation which provides site localization similarity as humans or subcutaneous, intraperitoneal, or intravenous inoculation (Bosma and Carroll 1991; Fujiwara 2018; Koo et al. 2009; Aartsma-Rus and van Putten 2019; Eswaraka and Giddabasappa 2017; Hirenallur-Shanthappa et al. 2017). Mice and rats are mostly preferred animals for this method because of easy handling, standardized, and homogeneity advantages.

The major disadvantages of these models are lacking tumor microenvironment except for orthotropic implantation, and the used animals need specialized environments such as specific-pathogen-free housing procedures both with autoclaved materials owing to their immunosuppressive situations.

"*What are the humanized models?*" Basically, the immunodeficient animals are engrafted with human cells or tissues, and these xenotransplanted parts physiologically act as in the human body. Various humanized mice have been generated up to date. Fundamentally, SCID mice, which lack of T and B lymphocytes, are engrafted with human peripheral blood mononuclear cells, human $CD34^+$ hematopoietic stem cells, or human fetal thymus and liver cells (Bosma and Carroll 1991; Fujiwara 2018; Eswaraka and Giddabasappa 2017; Hirenallur-Shanthappa et al. 2017). Humanized animals are becoming keystones not only for cancer but also in whole biomedical research areas.

To evaluate the immune response in order to advance cancer immunotherapy or immune response research, syngeneic – allograft, GE, or humanized models are suitable models (Li et al. 2017; Koo et al. 2009). Despite syngeneic models are cheaper than GE or humanized models, species-specific differences could give rise to translational failure.

Ethics in Animal Experimentation

Because of increased sensitivity for experimental usage of the animals, the ethical rules and limitations and end points of research were determined. After the introduction of the 3R (**R**eplacement, **R**eduction, and **R**efinement) principles by Russell and Burch in 1959 (Russell and Burch 1959), to date, we discuss the expansion of the "**R**"ules. The "R" concept (Table 2), enhancing 3R principles to 5R, includes "**R**igour" or "**R**obustness," and "**R**eproducibility" (Russell and Burch 1959 (as reprinted 1992); Kitano 2004; Obrink and Rehbinder 2000), and could be prolonged by new rules (e.g., t**R**ansparency, **R**esponsibility, etc.) to 7R (Lee et al. 2020; Tannenbaum and Bennett 2015), establishing the research culture that includes standardization of experimental animal usage. However, humanity is the first thing to keep in mind before handling animals to develop as the experimental model.

The major goal of animal usage in biomedical research is to achieve the translation ability to human and animal medicine. Thus, translational research is a bridge which comprises "*bench-to-bedside*" by means of the application of basic research to clinical utilization of both human and animal medicine (Cohrs et al. 2015). With the aim of the translational research, choosing the best animal model means finding the best matching organism with human (Mak et al. 2014). Related to recent reports, "*translational failure*" is a serious disadvantage in clinical trial phases and waste of the majority (Ledford 2011; Hackam and Redelmeier 2006). Hence, additionally to the 3R principles, rigor and reproducibility rules have to be essential for robust the obtained data (5R) and avoid the researcher-based prevention of negative results publication behavior, transparency and responsibility rules (7R) are indispensable for translational research.

Table 2 The "R" concept in biomedical research

			"**R**"ules	Definitions
7R	5R	3R	**R**eplacement	Primarily, choice alternative methods, e.g., mathematical and computer models (in silico), tissue culture systems (ex vivo), cell culture (in vitro). If you need a living organism (in vivo), choose insentient (nonsentient) primitive models (metazoan endoparasites, plants) or minimize the stress, pain by anesthesia and analgesia, do not harm, and maximize the animal welfare in the higher organisms
			Reduction	Principally, minimize animal usage through statistical limitations by obtaining reliable data to reduce the animal numbers and increase the obtained information. During the planning period, design the experiment in line with state-of-the-art knowledge; choose the right animal to model the research, adjust statistical methods, and determine the minimum sampling size to obtain reliable data
			Refinement	The term "well-being" could be defined as basically unstressed, feeling safe, maintaining normal behavioral and physiological conditions as animal welfare. Biological requirements and husbandry conditions such as eating, drinking, socializing, day/night cycle have to be maintained, and the researchers have to know the physiologic and behavioral requirements of selected model animals. Determine the limitations and cut-off situations to inhibit pain, fear, stress, and prevent inhuman applications
		or	**R**igor	The rigorousness of animal experimentation onsets with the experimental design by using vigorous scientific methods, robust and objective analysis, and detailed result transparency. This includes consulting with experts (veterinarians, biostatisticians, etc.) before the experimental period and sharing the raw data with the editor, reviewer(s), and readers in the publication period. Due to the translational challenge of animal research, the rigor and transparency directions, and new guidelines report officially (Shaffer 2021; Hewitt et al. 2017)
			Robustness	This term is defined as the quality of being strong and healthy. In terms of animal experimentation, robustness could be defined as the strength of the biological systems in the face of disturbing external (environment) or internal (physiological) conditions, and the quality of obtaining data taken from different laboratories with minimum variations, and translatability strength bench-to-bedside (Friggens et al. 2017; ten Napel et al. 2011). Robustness would lead to the clarity of complex systems and network analysis (e.g., signal transduction, disease mechanisms, therapeutic assays, etc.)
			Reproducibility	Rigor and robustness of research are tightly connected with reproducibility. It means repeating the capability of the same research and obtaining the same results during all repetition. This headline is the source of the big crisis among the same scientific experiments in different laboratories (Baker 2016). The origin of the reproducibility crisis in animal experiments is directly related to design methodology, age, sex, strain, and environmental conditions (von Kortzfleisch et al. 2020). The standardization of disease models, colony formation, and the collaboration among animal facilities could improve reproducibility

(continued)

Table 2 (continued)

			T**R**ansparency	Transparency includes detailed descriptions of methodology and evaluated data. To mirror reproducibility, robustness, and rigor, obtaining data in an animal experiment, transparency is the essential part (Aske and Waugh 2017; Hewitt et al. 2017). Sharing the raw data with the scientific community can improve the research methodology and translational capability by reducing the animal numbers and leading to state-of-the-art experiments
			Responsibility	The researchers/scientists have responsibility for using the animals in their experiments to ethics committees, editors, reviewers, as well as the global community. Hence, the researchers/scientists have to consist of the necessary qualifications such as animal usage license, physiological knowledge of model organisms, and high characteristics of ethics, morals, and in particular humanity

The Best Model Decision Algorithms of a Cancer Researcher

Here is the advice of some toolkits for cancer researchers to make the best decisions before taking action in their research.

The NC3Rs (National Centre for the Replacement Refinement & Reduction of Animals in Research) initiative is leading to new alternative methods for the replacement of animal usage in biomedical research (Singh 2012). Hence, the NC3Rs initiative contributes to the researchers by the ARRIVE (Animal Research: Reporting of in vivo experiments) guidelines to ensure the well-planning, rigorousness, and transparency of animal studies from study design, statistical methods to animal experimentation phases with the solidarity of an international working group (Percie du Sert et al. 2020). The ARRIVE guidelines have updated checklists not only for researchers but also for reviewers and journal editors.

Additionally, in cooperation with the Institute of Animal Technology, the animal technicians have supported the web-based training resources for animal research, which could be helpful for junior researchers related to various issues such as ethics, welfare, legislation, handling, and care of animals (RAT 2021).

Last but not least, Norecopa (Norway's National Consensus Platform for the advancement of the 3Rs) provides another web-based tool and guidelines for stakeholders of animal research namely PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) (Smith et al. 2018).

Conclusion

In conclusion, without a doubt, there are no certain models in cancer research and no perfect experimental design. Therefore, researchers must begin with a better plan and design the wisdom of their studies. The situation is serious, but not hopeless because of the researchers' websites, which have some artificial intelligence-based web instruments that are powerful tools for better scientific planning and design.

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