Validation of Animal Models

12

Umamaheswari Subramanian

Abstract

Animal research provides a major contribution to the discovery of new compounds and its mechanism of action. It also deals with the pharmacokinetics profile and determination of safe dose of a compound which is to be tested in humans. There is a necessity to choose an appropriate animal model for preclinical research in order to carry out a clinical trial. Research can be performed on already existing validated animal model or by validating a newer model. Validation criteria of an animal model changes from one to another based on the purpose of the model (fit-for-purpose). Face validity, predictive validity and construct validity ensures the closeness of the animal model to humans. In addition to these validity, few more criteria have been added to assess and optimise the animal model, i.e. epidemiology, symptomatology, natural history, end points, genetics, and biochemical parameters, pharmacological and histological features. There is no single animal model which can satisfy all types of validity for any disease. Even though shortcomings are inevitable, these models pave way for the safer research study in humans. One can choose an animal model closer to an ideal one. Thus validation plays a crucial role in translation of animal research to humans.

Keywords

Predictive · Face and construct validity · Animal model validation

U. Subramanian (✉)
Department of Pharmacology, Sri Venkateshwaraa Medical College Hospital & Research Centre, Puducherry, India

M. Lakshmanan et al. (eds.), *Introduction to Basics of Pharmacology and Toxicology*, https://doi.org/10.1007/978-981-19-5343-9_12

12.1 Introduction

Animal research provides a major contribution to the discovery of new compounds and its mechanism of action. It also deals with the pharmacokinetics profile and determination of safe dose of a compound which is to be tested in humans. Various animal species have also been used to study the disease pathogenesis. Animal welfare organisations have made the regulatory bodies in the field of animal research to follow stringent ethical guidelines while handling them. 3R has been followed as a guideline (Reduce, Replace and Refine).

12.2 Animal Model

One of the definition for animal model has been given by Held based on Wessler's original definition: "a living organism in which normative biology or behaviour can be studied, or in which a spontaneous or induced pathological process can be investigated, and in which the phenomenon in one or more respects resembles the same phenomenon in humans or other species of animal."

12.2.1 History –Animals in Research

The use of animals for purposes like understanding the human physiology date backs to ancient period. Till date it is being used judiciously for the betterment of human life (Fig. 12.1).

12.2.2 Classification of Animal Model

Animal models are classified based on various factors (disease -course, symptoms) (Tables 12.1, 12.2, and 12.3).

12.2.3 Utilization of Animal Models in Scientific Field

The ultimate use of animal models is to deal with the translation of the data obtained from the same to humans for the better health care. Extrapolation of the results from one species to the other is based on the evolution and the morphological and physiological similarity. The toxicity data obtained through animal models are used to determine the safer dose in humans. Certain information from these models do not get translated because of lack of relevance in humans. Significant contribution have been achieved by animal models to research and development:

- Type I diabetes treated with insulin - first demonstrated in the dog (Banting and McLeod)

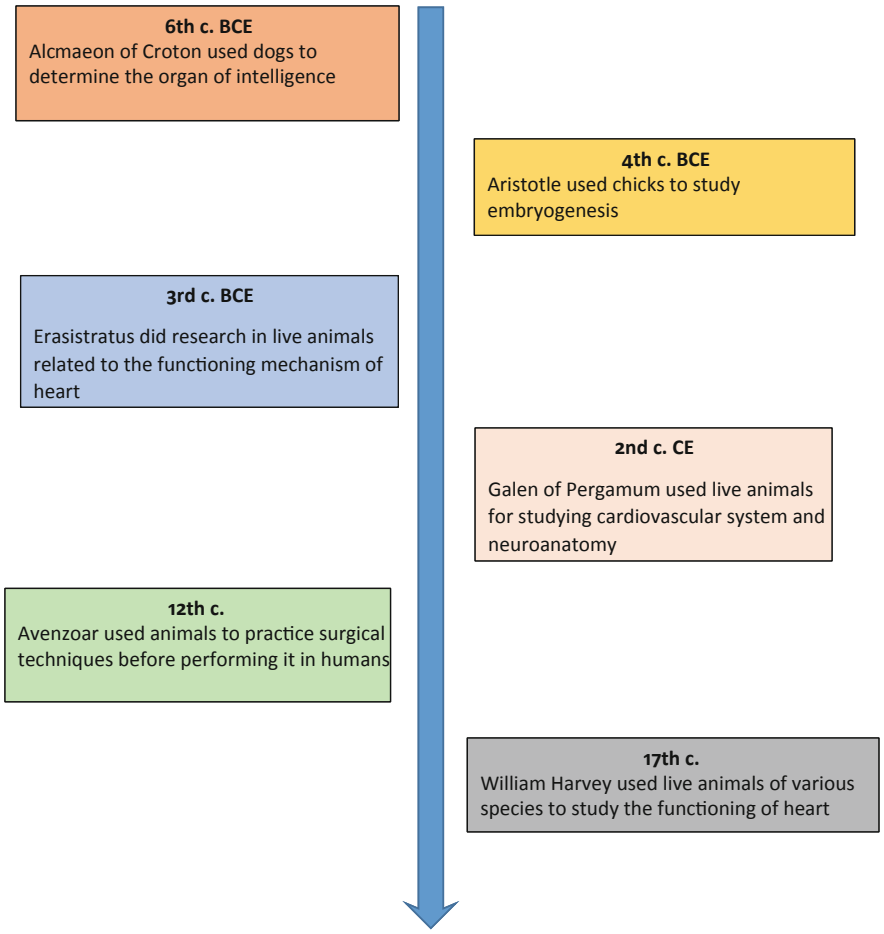

Fig. 12.1 Timeline of research activities using animals

Table 12.1 Types of animal models based on research design

S. No.	Model	Aim
1	Exploratory	Animal model are used to explore the fundamental mechanism(cellular, organ level) and identify it as normal or abnormal
2	Explanatory	Animal models are used to break down and understand the complex functional mechanism
3	Predictive	Animal models used to quantify the effect of treatment(curative) and also the toxic nature if any

- Evaluation of ex vivo, in vitro, and *in silico* models to attain better validation of extrapolated data from animal models
- Experimental design and methodology—crucial role to get valid extrapolation

Table 12.2 Different types of animal models based on symptomatology and course of the disease

S. No.	Model	Description
1	Homologous animal model	Symptoms and course of disease in animal model is same as that of humans
2	Isomorphic animal model	Symptoms of the disease in animal model is same as that of humans but causative factor of disease is different
3	Partial animal model	It helps to reproduce only a certain part of human disease and treatment for the same

Preclinical research is a preliminary level where the drug is studied before entering the clinical phase.

12.2.4 Ideal Animal Model

Any animal model should have these following characteristic features:

1. Appropriateness to human disease
2. Translation to humans
3. Genetic uniformity or closer to humans
4. Cost and availability- cheap & easily available
5. Generalizability of the results
6. Ethical consideration should be addressed

12.3 Validation of Animal Model

Validation means the relation between test score and its quality to measure. It should cover internal validity (replicability), generalizability, predictive and construct validity. There is a necessity to choose a proper and appropriate animal model for preclinical research in order to carry out a clinical trial which will eventually yield results through which humankind can be benefitted. Hence selection of the same requires validation. Research can be performed on already existing validated animal model or by validating a newer animal model.

Animal model is being called so only when it has been validated whereas it is called as putative animal model if it is yet to undergo validation. Validation criteria of an animal model changes from one to another based on the purpose of the model (fit-for-purpose). It is impossible for a single animal model to achieve validity with all criteria. In order to obtain the maximum number of validity criteria, optimal combination of models should be considered.

Criteria for valid animal model includes:

Table 12.3 Animal models- Advantages & Disadvantages

S. No.	Model	Example	Advantage	Limitations
1	*Induced (experimental) models:* Healthy animals induced to produce a specific disease	Diabetes mellitus induced with encephalomyocarditis virus.	Choice in selecting the species	Majority of these models are either isomorphic or partial
2	*Spontaneous (mutant) models*: The animal model with natural genetic variation/ mutation	Athymic nude mouse	1. Phenotypic similarity with respect to disease between humans and animal (face validity) 2. This model plays significant role in the development of therapeutic regimen in humans	Genetic impairment will lead to various compensatory responses which will differ between human and animals
3	*Genetically modified models:* Genetic modification in the animal- transgenic animals	Use of mutagen (ethylnitrosourea) to induce genetic mutation in animals	1. Able to identify the cause and genetic involvement of certain diseases 2. Able to study about gene-gene interaction, gene-environment interaction and the effects produced due to alteration in genetic pathway	Phenotyping of the animals found to be difficult
4	*Negative models:* Animal models which remain unresponsive towards a pathogen which causes infection in human	Gonococcal infection in rabbit (where rabbit do not have susceptibility)	Provides physiological basis for resistance and its mechanism	
5	*Orphan models:* Disease occurs in animals but not yet discovered in humans (but can be identified later)	1. Bovine spongiform encephalopathy 2. Feline leukemia virus.	Useful information provided by the orphan models in case humans get infected by the same infection.	

1. Face validity
2. Construct validity
3. Predictive validity

The above mentioned validity criteria comes under the broader component called external validity.

12.3.1 Face Validity

The symptoms induced in animal model should match the symptoms of humans, i.e. phenotypic similarity. Example Type I Diabetes mellitus in humans and insulin requiring animal (BB rats). These two conditions respond well to the insulin therapy. Most of the spontaneous animal models exhibit face validity.

12.3.2 Construct Validity

Presence of homology between the human and animal model on genomic level is called construct validity. Transgenic disease models comes under this category. Animal model with this validity will give inputs about the occurrence of a disease condition with the effects of genetic change and interaction of gene with the environment.

12.3.3 Predictive Validity

It shows how much an animal model can predict an unknown aspect of the human disease or its therapy. It major contribution is by assessing the cure of a disease. It do not go in for specific cause of a disease and its treatment.

All these three criteria were proposed by Wilner. These three criteria falls under external validity and provides basis for generalisation of the results obtained from animal studies to humans. Belzung and Lemoine proposed a nine validity criteria in case of models related to psychiatric disorders. Tricklebank and Garner suggested other criterias in addition to Wilner's—internal validity (third variable influencers), external validity (results can be generalized), convergent validity, discriminant validity. It is mostly not possible to have an animal model with all these above criteria/validity, but researchers insist the presence of predictive validity which is the most important one.

Any animal model is said to be valid if it resembles humans with respect to the above said aspects as depicted in the figure (Fig. 12.2).

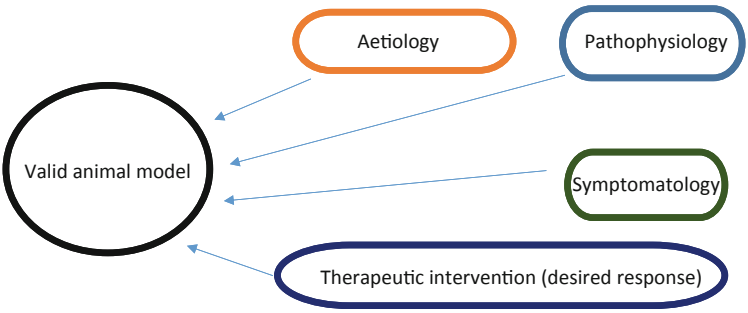

Fig. 12.2 Significant components—Valid animal model

12.3.4 Internal Validity

Internal validity means the results or outcomes among animals (different treatment groups) varies only with respect to the intervention. If so those animal models are considered to be with adequate internal validity. Internal validity may be reduced if there is any flaw in the design of animal model and conduct of the study. Certain bias (selection bias, performance bias, attrition bias and detection bias) will affect the internal validity.

Reliability—consistent results (similar experimental condition).
Replicability—reproducibility.
High replicability & reliability—good internal validity.

12.3.5 Factors Affecting Internal Validity

1. Rearing conditions
2. Housing conditions
3. Social hierarchy
4. Gender
5. Age
6. Time of testing
7. Day-night cycle
8. Health condition of research animal
9. Calibration of research equipment

These factors need to be addressed to increase the internal validity (Table 12.4).

Animal models can either be holistic or reductionist. Holistic model- the model as a whole (symptoms, behaviour, underlying mechanism, etc.) is comparable to human targets. Reductionist model- any specific feature of the animal model is alone comparable to the human targets.

Table 12.4 Different validity of animal models

S. No.	Validity of animal model	Description
1	Discriminant validity	The model proposed is based on the divergent property where the measured parameter do not relate as expected. i.e. The concept and its correlating factors diverge
2	Mechanistic validity	As such, the behaviour resemblance or similarity between the animal model and human targets is not enough but the underlying mechanism for those behaviour should be identical
3	Convergent validity	The model proposed is based on the convergent property where the measured parameter do relate as expected. i.e. The concept and the variables measured are closely related
4	Biomarker validity	The animal model and the human targets may have variations in the biomarkers due to the difference in the species, but if both of them produce the same symptoms and also matches the underlying mechanism, then it is considered as valid animal model
5	Target validity	Target being studied in the animal model should be comparable to that of human in terms of mechanism/function

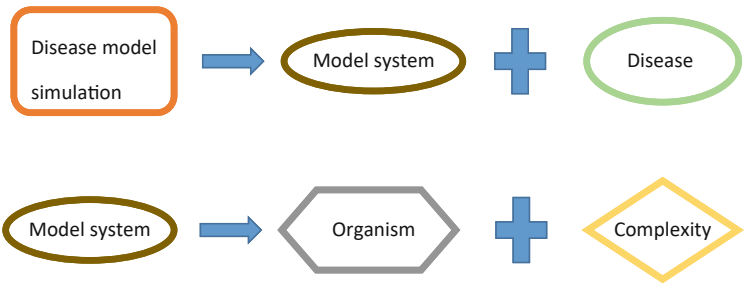

Fig. 12.3 Disease model simulation and model system

12.4 Process of Developing an Animal Model

Developing an animal model should be done by a series of steps in a meticulous manner. Researcher should have the knowledge about certain terminologies before going into this process (Figs. 12.3, 12.4, and 12.5).

12.5 Validation Process for Predictive Validity

Among face validity, predictive validity and construct validity, main focus of validation process is on predictive validity. The following steps are involved in the validation process:

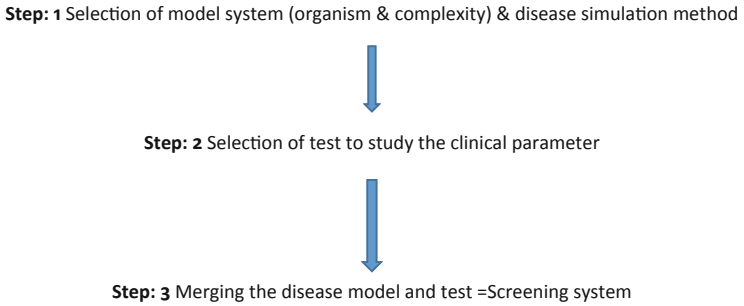

Fig. 12.4 Valid screening system reflects predictive validity which is a crucial component for an appropriate animal model. It is obtained by stepwise approach as depicted in the figure

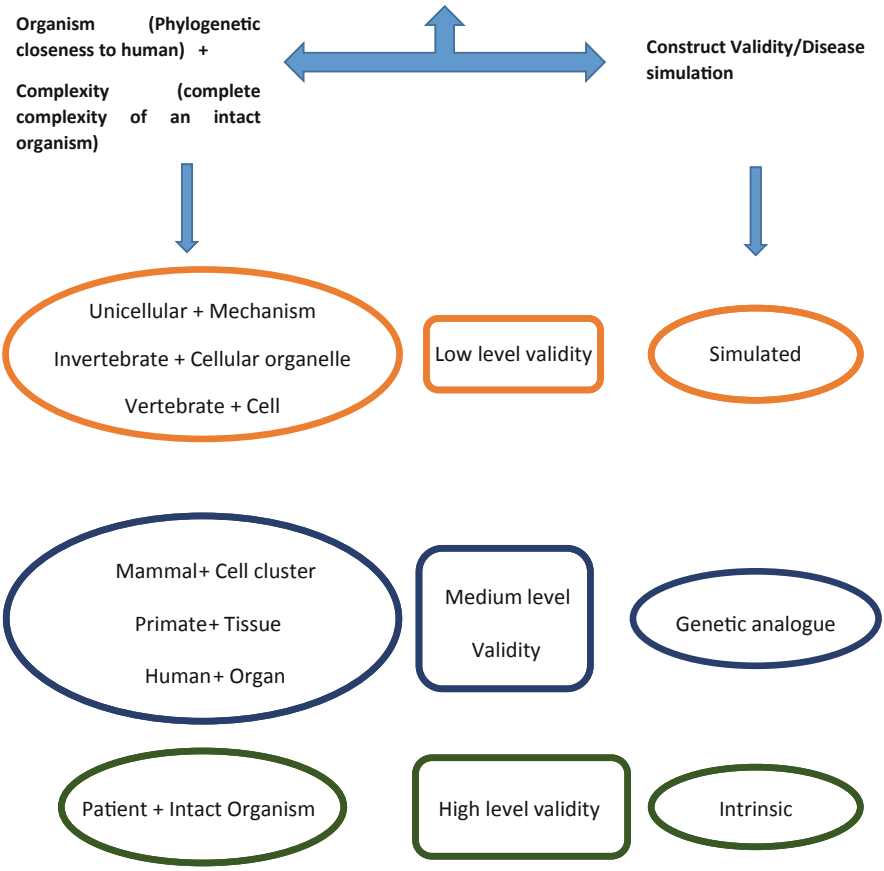

Fig. 12.5 Disease model validity (organism with complexity + construct validity)

1. Test development: Development of diagnostic/therapeutic intervention to predict the outcome of the same in a specific disease
2. Pre-validation: Test is performed in two or more laboratory (relevance & reliability)
3. Validation: Test must be relevant and also reliable for more than one purpose. Data analysis should be done followed by further evaluation.
4. Independent assessment: The study data and conclusion must be published after peer reviewing the same and should be done by the independent panel.
5. Regulatory body acceptance

This validation process can also be performed in a retrospective manner, not necessary to be unidirectional all the time. But retrospective method found to show less reliability.

12.6 Framework to Identify Models of Disease (FIMD)

- This framework was done by Ferreira GS et al.
- Initial step for validation of animal models
- Identify the aspect in humans which need to be simulated in animals
- Selection of appropriate disease model is necessary which predicts (outcome in humans) accurately
- Parameters significant for validation of animal model should be identified
- Eight domains were included to select an optimal animal model (Fig. 12.6)

Level of Validation:

1. Insufficient validation (0–40% of definitive answer)
2. Slightly validated (41–60% of definitive answer)
3. Moderately validated (61–80% of definitive answer)
4. Highly validated (81–100% of definitive answer)

Definitive answer—All answer except unclear for the above questions in each domain (Table 12.5).

12.7 Evaluation of Valid Animal Model

Animal model can be evaluated by comparing it with the data acquired from humans. This happens only if the research has progressed from preclinical to clinical trials. Repeatability and reproducibility can be analysed in this evaluation (Tables 12.6 and 12.7).

Discrepancy to clinical state is 3 if all three components (organism, complexity & disease simulation) shows discrepancy when compared with human disease

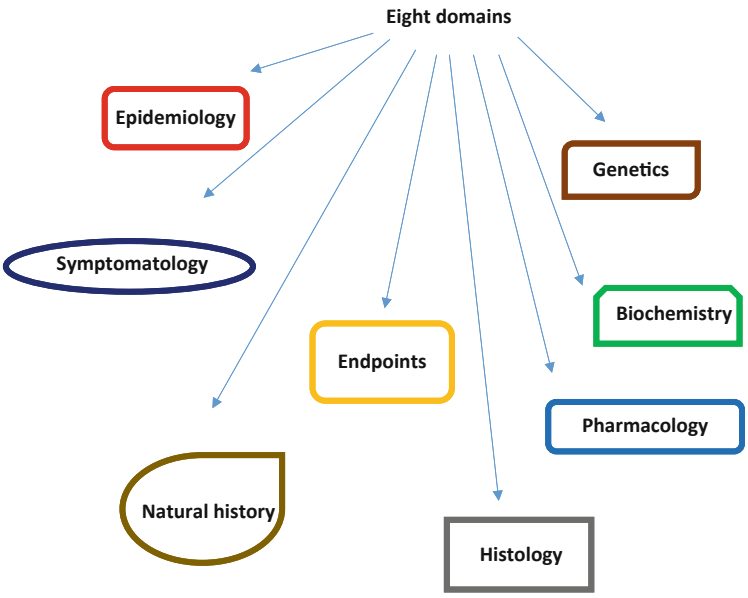

Fig. 12.6 Domains for framework to identify models of disease (FIMD)

condition, similarly discrepancy to clinical state is two if any of the two components did not match the human disease.

12.8 Limitations of Animal Model

- Certain clinical endpoints (biomarkers) are not reproduced with animal models hence following research process (preclinical study) is not achieved in few disease conditions.
- Quality of life cannot be assessed with the help of animal model.
- Ideal animal model do not exist.
- Experimental group with less number of samples exhibit insufficient power.
- Duration of follow up in animal models varies with the human.
- Disparity between animal and humans in the drug metabolic pathways.

12.9 Conclusion

Animal model need to be selected wisely based on the human disease condition and the predictive outcome. There are many animal models available for a single disease condition, appropriate model (proper validation) is selected based on the criteria of concern. Even though shortcomings are inevitable, these models pave way for the

Table 12.5 Weightage and questions to be raised under each domain for optimising animal model (FerreiraI et al. 2019)

S. No.	Question	Weightage
1	*Epidemiological validation:* 1. Is the model able to simulate the disease in the relevant sexes? 2. Is the model able to simulate the disease in the relevant age groups (e.g., juvenile, adult or ageing)?	**12.5** 6.25 6.25
2	*Symptomatology and natural history validation:* 1. Is the model able to replicate the symptoms and co-morbidities commonly present in this disease? If so, which ones? 2. Is the natural history of the disease similar to human's regarding 2.1 Time to onset 2.2 Disease progression 2.3 Duration of symptoms 2.4 Severity	**12.5** 2.5 2.5 2.5 2.5 2.5
3	*Genetic validation:* 1. Does this species also have orthologous genes and/or proteins involved in the human disease? 2. If so, are the relevant genetic mutations or alterations also present in the orthologous genes/proteins? 3. If so, is the expression of such orthologous genes and/or proteins similar to the human condition?	**12.5** 4.17 4.17 4.16
4	*Biochemical validation:* 1. If there are known pharmacodynamic (PD) biomarkers related to the pathophysiology of the disease, are they also present in the model? 2. Do these PD biomarkers behave similarly to humans? 3. If there are known prognostic biomarkers related to the pathophysiology of the disease, are they also present in the model? 4. Do these prognostic biomarkers behave similarly to humans?	**12.5** 3.125 3.125 3.125 3.125
5	*Aetiological validation:* Is the aetiology of the disease similar to humans?	**12.5** 12.5
6	*Histological validation:* Do the histopathological structures in relevant tissues resemble the ones found in humans?	**12.5** 12.5
7	*Pharmacological validation:* 1. Are effective drugs in humans also effective in this model? 2. Are ineffective drugs in humans also ineffective in this model? 3. Have drugs with different mechanisms of action and acting on different pathways been tested in this model? If so, which?	**12.5** 4.17 4.17 4.16
8	*Endpoint validation:* Are the endpoints used in preclinical studies the same or translatable to the clinical endpoints? Are the methods used to assess preclinical endpoints comparable to the ones used to assess related clinical endpoints?	**12.5** 6.25 6.25

safer research study in humans. One can choose an animal model closer to an ideal one. Thus validation plays a crucial role in translation of animal research to humans.

Table 12.6 Scoring system—validation of animal model (Sams-Dodd et al., 2006)

S. No.	Criterion		Score
1	Species	Human	4
		Non-human primate	3
		Non-human mammal	2
		Non-mammal	1
2	Simulation of disease	True	4
		Complex	3
		Pharmacological	2
		No	1
3	Face validity	More than 1 core symptom	4
		One core symptom	3
		One symptom	2
		No	1
4	Complexity	In vivo	4
		Tissue	3
		Cellular	2
		Sub-cellular/molecular	1
5	Predictivity	Graded for all pharmacology principles	4
		Graded for certain pharmacology principles	3
		All or none for certain pharmacology principles	2
		No or not shown	1

Table 12.7 Animal model validity based on drug screening system (Sams-Dodd et al., 2006)

S. No.	Drug screening method	Organism	Complexity	Disease simulation	Discrepancies to clinical state
1	Receptor binding assay	Mammal	Mechanism	None	3
2	Animal tissue	Mammal	Tissue	Artificial	3
3	Isolated organ	Mammal	Organ	Artificial	3
4	Animal disease model	Mammal	Intact organism	Artificial	2
5	Patient tissue	Patient	Tissue	True disease	1
6	Transgenic animal	Mammal	Intact organism	True disease	1(familial) 2(sporadic)
7	Human patient	Patient	Intact organism	True disease	0

Bibliography

Andersen ML, Winter LMF. Animal models in biological and biomedical research – experimental and ethical concerns. An Acad Bras Cienc. 2017;91(Suppl 1):1–13.

Bailey J, Balls M. Recent efforts to elucidate the scientific validity of animal-based drug tests by the pharmaceutical industry, pro-testing lobby groups, and animal welfare organisations. BMC Med Ethics. 2019;20(16):1–7.

Denayer T, Stohr T, Van Roy M. Animal models in translational medicine: validation and prediction. New Horiz Transl Med. 2014;2:5–11.

Ericsson AC, Crim MJ, Franklin CL. A brief history of animal modeling. Mo Med. 2013;110(3): 201–5.

Ferreira GS, Veening-Griffioen DH, Boon WPC, Moors EHM, Gispen-de Wied CC, Schellekens H, et al. A standardised framework to identify optimal animal models for efficacy assessment in drug development. PLoS One. 2019;14(6):1–17.

Ferreira GS, Veening-Griffioen DH, Boon WPC, Moors EHM, Meer PJK. Levelling the translational gap for animal to human efficacy data. Animals. 2020;10(1199):1–13.

Hau J. Animal models. In: Hau J, Hoosier GLV, editors. Handbook of laboratory animal science. 2nd ed. Boca Raton: CRC Press; 2003. p. 1–11.

Hau J. Animal models for human diseases. In: Conn PM, editor. Sourcebook of models for biomedical research. Totowa, NJ: Humana Press Inc; 2008. p. 3–8.

McGonigle P, Ruggeri B. Animal models of human disease: challenges in enabling translation. Biochem Pharmacol. 2013;87(1):1–39.

Pound P, Ebrahim S, Sandercock P, Bracken MB, Roberts I. Where is the evidence that animal research benefits humans? BMJ. 2014;328:514–7.

Robinson NB, Krieger K, Khan F, Huffman W, Chang M, Naik A, Yongle R, Hameed I, Krieger K, Girardi LN, Gaudino M. The current state of animal models in Research: a review. Int J Surg. 2019;10:1–21.

Sam-Dodd F. Strategies to optimize the validity of disease models in the drug discovery process. Drug Discov Today. 2006;11(7):355–63.

Sjoberg EA. Logical fallacies in animal model research. Sjoberg Behav Brain Funct. 2017;13(3): 1–13.

Varga OE, Hansen AK, Sandoe P, Olsson IAS. Validating animal models for preclinical research: a scientific and ethical discussion. ATLA. 2010;38:245–8.

Worp HB, Howells DW, Emily Sena ES, Porritt MJ, Rewell S, Collins VO, et al. Can animal models of disease reliably inform human studies? PLoS Med. 2010;7(3):e1000245.