



# Alternatives and Refinement for Animal Experimentation in Cancer Research

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**Abstract.** Globally cancer is a major public health issue and is a second biggest cause of deaths. Although animal models have limitations in terms of predictive and translational value to humans, they have played a major role in understanding this disease and anticancer drug discovery. In cancer research the most commonly used animal species are mice, rats, hamsters, rabbits, Guinea pigs, fish and amphibians. Use of different cell lines in tissue culture system offers a great relief from use of animals at the same time provide important clues before embarking the animal experiments. Use of spontaneously developing tumor models are encouraged rather producing diseases in the animals. Beside the alternatives, refinement also plays important role in reducing the animal usage in cancer research. However, cancer research use whole body system to evaluate the new strategies for diagnosis and treatment of cancer. This paper highlights the available alternatives and refinements for animal experimentation. A positive note is that if coupled with the refinements, even a minimised number of animal usage shall also yield the meaningful and acceptable results.

**Keywords:** Cancer research · Animal models · Alternatives · Refinement

## Introduction

Cancer researcher plays an important role in investigating new modalities of diagnosis and treatment. This includes discovering the faulty genes and molecules that cause cancer, investigating how the disease grows and spreads, developing and testing new treatment and tests, and exploring how our immune system can help fight tumours. Literature surveys indicate that amongst various species, rodents have been extensively used in biomedical research. The laboratory mouse and rat is a common experimental model, in part because of well-defined strain genealogies, standardized mapping tools, and the availability of sophisticated genetic monitoring technologies. When animals are used for research, it is the moral duty of the scientist to avoid or minimize discomfort, distress, and painful situations. The regulations and policies help to ensure animals are treated humanely. If a procedure involves more than momentary or slight pain or distress, it must be performed using appropriate pain relieving drugs (e.g. sedatives, analgesia or anesthesia) [1].

Knowledge gained through use of animals in research is still improving the life of not only humans but domestic as well as pet and wild animals too. Discovery of

antibiotics like penicillin, erythromycin, tetracyclin etc. would have not been possible without involvement of animal testing.

Further the discovery of Nude and SCID mice model have made significant improvement in these models and have made it possible to identify clinically efficacious agents through anticancer research and testing. These models are said to be a 'Workhorse' of the pharmaceutical industry. These models have not only helped in characterization of cancer cell lines but also helped us in understanding metastatic processes and efficacy of the newly developed anticancer drugs [2].

Cancer researchers need predictive and cost effective preclinical models that more accurately predict the response of human cancer to chemotherapy. Experimental models of human cancers are important in reconstructing the events that occur in human patients with cancers. A variety of factors decides the choice of tumor and model.

## Alternatives in Cancer Research

Scientists use many ways to try to replace animals used in research. These include using cell cultures, computer modelling and human studies. Researchers must attempt using these techniques if they would be as effective as using animals.

Responses to chemicals are complex and difficult to accurately assess using only biochemical or cell-based (*in vitro*) systems or computer models. No single *in vitro* test method can be employed to serve all regulatory needs for a specific testing area. Mechanistically based alternative approach may provide promising opportunity for assessing hazards of regulatory concern. Adverse outcome pathways (AOPs) are expected to provide insight into the biological relevance, reliability, and uncertainties associated with the results from *in silico*, *in chemico* and *in vitro* approaches for regulatory use. This provides the biological context and supporting weight of evidence to facilitate the interpretation of such alternative data [3]. Severe eye irritants and substances that could cause allergic contact dermatitis is the only option where major progress has been made in reducing and replacing animal use [4]. For other hazards that can cause cancer or birth defects, development of *in vitro* tests that reliably identify hazards is more difficult because of the number of different mechanisms involved in these complex biological processes. However, some lower vertebrate offers the best alternatives in cancer research.

Zebrafish, *Danio rerio* have become a popular model for studying developmental processes and human diseases. Fish and human solid tumors share a high degree of histological similarity and therefore have been used extensively as model for human cancers. Zebrafish also form spontaneous tumors with similar histopathological and gene expression profile as human tumors [5]. Zebrafish have proved to be useful for use in cancer research over the last decade. There are several long-standing methods for establishing a cancer model in zebrafish, including carcinogenic treatment, transgenic regulation, and the transplantation of mammalian tumor cells. Some of the analysis of metastasis pathway is conducted in tightly controlled *in vitro* cell system usually involving up-regulation or down-regulation of genes. These assays include trans-well motility, wound healing, invasion and hanging drop assays. These assays do not answer

issues of intravasation of tumor cells into blood vessels or extravasation in to distant organs. In order to study these processes, there is no alternative to *in vivo* systems. However, zebrafish can offer these assays as an alternative to rodents or other vertebrates animals to study the human tumor angiogenesis or metastasis. Because of the transparent in nature, Zebrafish offer excellent features to study tumor angiogenesis as well as metastasis [6, 7]. Reports have also shown that primary human cancer cells can metastasize in fish and this ability can be used to predict metastatic potential in a clinical setting. Zebrafish has been used to study the metastasis of lymphoblastic leukemia, melanoma, breast tumor, prostate cancer, rhabdomyosarcoma, testicular cancer, colon cancer, neuroblastoma and pancreatic tumors [8–13].

Organisms like Hydra have been used as a model for regeneration, embryogenesis, testing the water pollutant eco-pollutant and environmental genomics [14, 15]. However, it remains to be seen whether they can also be used explored for studying the carcinogenesis.

Efforts are also targeted towards use of ‘artificial tumor’ tissue grown from stem cells or a combination of tumor cells and tumor stromal cells etc. [16, 17]. Full-thickness skin models can be used to test the effect of substance in question on the skin tissues. However, since cancer research needs a complete body system for testing, this model may be of little hopes.

Use of spontaneously developing tumor models like leukemia, lung cancer, brain cancer and breast cancer caused by MMTV or any other aetiology are encouraged in lieu of producing these diseases in the animals.

### **3R's**

WMS Russell and RL Burch originated the concepts of replacement, reduction, and refinement, which they published in their book in 1959 entitled “*The Principles of Humane Experimental Technique*” [18]. Russell and Burch proposed that if animals were to be used in experiments, every effort should be made to ‘Replace’ them with non-sentient alternatives, to ‘Reduce’ to a minimum number of animals used, and to ‘Refine’ the experiments so that they caused the minimum pain and distress.

Replacement alternatives advocate methods to be used in achieving scientific results without conducting experiments on animals. There is a direct correlation between larger number of animals used to higher overall costs and animal suffering. Therefore, the number of animals used should be kept to a minimum that would be consistent with the research objectives. Refinement alternatives include methods that alleviate or minimize potential pain and distress thus emphasizing on animal well-being.

### **CPCSEA**

The Institutional Animal Care and Use Committee (IACUC) or Institutional Animal Ethics Committee (IAEC) is legally required to oversee all animal care and use activities conducted at their institution. IACUC/IAEC is also required to set high

ethical and welfare standards for using the animals. In India the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), established under the Ministry of Environment, Forests and Climate Change has been instrumental in avoiding the animal usage or if not possible, to reduce the number of animals used in experimentation. At the local level, IACUC/IAEC are mandated to ensure that: the number of animals used for the research in each groups are enough to yield statistically valid results; appropriate species of animal is used for the project; humane experimental endpoints have been established and appropriate methods of euthanasia are being utilized [19]. The CPCSEA integrate the 3R's in all R&D processes and procedures. It gives emphasis on review of animal models on a continuous basis for replacement with alternatives. It also promotes use of human cells and tissues instead of living animals, wherever possible. The CPCSEA is charged with reviewing and approving all research and testing activities in India that involve animals before scientists begin their experiments to ensure that there are no alternatives to using animals, that research is not being unnecessarily duplicated, and the experiment is relevant to human or animal health and will be for the betterment of the society.

## Refinement in Cancer Research

For more than a half century mice have been the primary species in which experimental cancer chemotherapy have been tested [20]. Cancer drug screening program of the US National Cancer Institute (NCI) started with the ascitic and then with solid tumors. This program used conventional mouse models like C57BL/6, Swiss, BALB/c and DBA/2 strains. After the discovery of the Nude and SCID mice, widespread use of human tumor transplantation for anticancer research and testing was made possible [21, 22]. Mice and rats often develop benign and malignant cancers spontaneously. The incidence may vary from strain to strain. These spontaneous tumors do not always metastasize to different organs or have mild local tissue invasion and therefore the incidence is very rare. However, majority of the data generated on the metastatic behavior of cancer is reported to be derived from studies in rodent models only [23, 24].

'Specialized' models like transgenic, knock out, Nude, SCID and hairless SCID mouse models have played a central role in cancer research. Models like SCID beige, NIH-III, NOD-SCID, SCID beige, NSG, NOG and Rag-1 and 2 mutation mouse model which lack T-, B- as well as NK cell activity are the next level of 'super specialized' models, which supports not only the hetero-transplantation but also the metastasis studies.

Use of T- and B-cell deficient animals as shown by the use of flow cytometry; use of rodent pathogen-free animals; use of genetically proven animal models; use of appropriate number of cells and volume for injection; use of appropriate route of injection; and use of properly genotyped/phenotyped animal models helps to reduce the number of animals used for the respective research and also yield authentic and reproducible results. Use of spontaneously developing tumor models such as mammary gland tumors in C3H/J mice, leukemia in AKR mice, lung cancer in A/J mice and brain tumor in *Ptch* or *Smo* knockout mice is one of the refinements. New tumors or tumor-

like growth found in the laboratory animals can be a potential material to study the disease processes. Spontaneously developing papilloma incidence has been reported in Nude mice by Ingle et al., 2011 [25]. These are characterized to be caused by mouse papillomaviruses. This model has been exploited and characterized fully for its cause, genomic analysis, molecular diagnosis and sequencing of the virus. [26–29].

In order to ascertain the absence of T- and B-cells in the immune-compromised animals, assessment of these cells by flow cytometry is the best method [30]. This can be done by measuring the CD3 antibodies for T-cells and CD19 antibodies for B-cells. Additionally, subsets of CD3 can also be checked by including CD4 and CD8 antibodies.

## Conclusion

Use of animals remains unavoidable for the time being for the development of new and more effective methods for diagnosis and treating diseases that affect both humans and animals. However, it is our moral duty to see that the use of animals is either replaced or at least minimized to the extent to get the meaningful as well as reproducible and acceptable results. With its own advantages and disadvantages, the zebrafish embryo as well as zebrafish tumor xenograft model can be very well used as a tool for investigating the cancer biology including neovascularization for drug discovery and gene targeting in tumor angiogenesis. With refinement of the quality of animals through genetic & microbiological testing, refining the volume and route of injection and use of spontaneously available tumor models, it is possible to achieve the same results in cancer research.

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