

# Of Mice and Humans: Are They the Same?—Implications in Cancer Translational Research

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Animal models have been instrumental in elucidating key biochemical and physiologic processes of cancer onset and propagation in a living organism. Most importantly, they have served as a surrogate for patients in the evaluation of novel diagnostic and therapeutic anticancer drugs, including radiopharmaceuticals. Experimental tumors raised in rodents constitute the major pre-clinical tool of new-agent screening before clinical testing. Such models for oncologic applications today include solid tumors raised in syngeneic fully immunocompetent hosts and human xenografts induced in immunodeficient mouse strains, and tumors spontaneously growing in genetically engineered mice represent the newest front-line experimental modality. The power of these models to predict clinical efficacy is a matter of dispute, as each model presents inherent strengths and weaknesses in faithfully mirroring the extremely complex process of human carcinogenesis. Differences in size and physiology, as well as variations in the homology of targets between mice and humans, may lead to translational limitations. Other factors affecting the predictive power of preclinical models may be animal handling during experimentation and suboptimal compilation and interpretation of preclinical data. However, animal models will remain a unique source of *in vivo* information and the irreplaceable link between *in vitro* studies and our patients.

**Key Words:** syngeneic xenogeneic tumors; orthotopic subcutaneous tumors; drug development; translational studies

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**Animal models have been essential in cancer research for obvious practical and ethical concerns associated with human experimentation.** In fact, the requirement for animal-model studies as a prerequisite to human clinical trials was codified as the Nuremberg Code soon after World War II. This concept has been adopted by law both in the United States and in Europe, with the respective drug agencies (Food and Drug Administration and European Agency for the Evaluation of Medicinal Products) specifying that animal studies

should precede human tests for approval of new biomedical products, including new radiopharmaceuticals (1–3). Accordingly, newly developed radiolabeled vectors for scintigraphy and radionuclide therapy of cancer have to be studied in laboratory animals before human administration (4–8). Animal studies can provide invaluable pharmacologic and toxicologic data and may predict the clinical efficacy of new compounds. Furthermore, animal studies allow environmental and genetic manipulation rarely feasible in humans.

Generally, rodents are being used for such studies, with mice being the mainstay because they are small, breed readily, can be genetically modified rather easily, and are usually inexpensive. Because of their short gestation and life span, mice allow rapid breeding of a large number of animals and, consequently, the feasibility of many studies in a relatively short period.

Although mice and humans are at least 95% identical at the genomic level, this similarity obviously does not prevent their respective phenotypes from being very different. In fact, the literature is littered with examples of pharmaceuticals that show good results in mice but fail to provide similar efficacy in humans. In this review, our aim is not to suggest that the mouse is an invalid model for human studies. Clearly, with so many paradigms that translate well between the species, mouse models will continue to provide unique information. Rather, our aim is to improve the understanding of the applicability of laboratory animal data to human studies. Also, we will review reports that investigated shortcomings that influenced the translational value of animal studies.

In our research teams, we have ample experience with rat and mouse models for tumor scintigraphy and radionuclide therapy; the focus of this article will therefore be on such models for cancer research.

## ANIMAL MODELS IN CANCER RESEARCH

Among the earliest *in vivo* tumor models implemented in the 1960s were ascitic murine leukemia models. Soon afterward, research was directed toward modeling solid tumors into mice to provide the tools needed for screening

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a broader array of anticancer medicines (1–4). Table 1 summarizes the major mouse models used in anticancer drug research up to now, along with their major strengths and shortcomings.

In syngeneic models, mice bear tumors originating from their own species. Carcinogenesis is induced by chemical or surgical intervention; subsequently, material from this first tumor (either explants or cells) is introduced to naïve members of the same mouse strain. Advantages of syngeneic models are ease of implementation, availability of hosts at a low cost, reproducibility of experimental tumor histology and growth rate, and the simplicity of the statistical analysis required for data validation. In addition, the host retains full immunoreactivity, and tumor induction is not immunogenic. Furthermore, appropriate interaction of introduced malignant cell lines with host stroma elements is favored. However, syngeneic models often fail to adequately represent the human situation. As modern medicines are directed to specific cancer-residing targets, the homology between the mouse and human versions of target biomolecules may turn out to be a serious limitation for syngeneic models (9).

Alternatively, xenografts of human origin can grow in immunosuppressed hosts, such as in genetically manipulated athymic mice (*nu/nu*), or in severe combined immunodeficiency mice, which lack both humoral and cellular immune components. Tumor induction is usually triggered by injecting

human tumor material into the host either subcutaneously or orthotopically. Subcutaneous xenogeneic models have been the mainstay of anticancer drug development over the last 25 y, mostly because they are better predictors of drug efficacy in human tumors. Malignant cells are human and consequently express the human homolog of the target biomolecule. However, the microenvironment around the tumor is provided by the nonhuman host. Discrepancies may arise in tumor histology and intra- and peritumoral vasculature as a result of altered interaction patterns between the human tumor and the extracellular matrix of the murine host. These differences can be minimized when human tumors are directly transplanted from patients into mice, whereby the xenograft histology closely resembles the histology of the patient tumor. In this case, the tumor cells instead of the host stroma seem to dictate lesion architecture, molecular features are preserved, and existing human proangiogenic factors will “cooperate” with elements of the host peritumoral micromilieu in the process of neovasculture formation.

Subcutaneous xenogeneic models have dominated anticancer drug research as a result of their simplicity, reproducibility, and homogeneity in tumor histology and growth rates. In addition, a wide range of well-established human cell lines have long been available to researchers, and drug efficacy databases have been created over the years. Hosts are also widely available, albeit more costly than normal mice. In view of their immunodeficiency, mouse

**TABLE 1.** Mouse Models in Cancer Research: Characteristics, Strengths, and Shortcomings in Implementation and Clinical Efficacy Prediction

Type	Material	Site	Advantages	Drawbacks
Syngeneic	Chemical induction— injection of cells or explants from the same species	Predominantly subcutaneous; orthotopic	Immunocompetent hosts; availability of well- characterized cell lines; low cost; ease of implementation; reproducibility of tumor properties; preserved stroma–cancer cell interactivity; simple statistics	Often poor representation of human disease; sometimes lack of target-molecule homology between species
Xenogeneic	Injection of human cell lines or explants	Subcutaneous (unnatural site)	Availability of well-characterized cell lines; simple to implement; expression of human homolog of target; efficacy databases available; reproducibility; homogeneity in tumor characteristics	Immunosuppressed and nonhuman hosts; more costly and microbe-free animal housing; murine peritumoral milieu; different from human tumor histology
		Orthotopic (primary tumor source site)	Best mimicking of human carcinogenesis and metastatic patterns	Complex logistics; surgical skills; limited number of hosts; nonhomogeneity or nonreproducibility of tumor growth rates; problems with statistics
Genetically engineered	Expression of target or label—spontaneous carcinogenesis		Controlled cancer progression in selected organs; resemblance of human carcinogenesis; immunocompetent syngeneic host	Limited availability of hosts; restricted experience; cost; variations in tumor growth rates; demanding statistics; murine host stroma

hosts are susceptible to infections and need to be housed in a microbe-free environment. Still, the stromal component of model tumors remains murine and, most importantly, tumors grow at an unnatural site.

In the orthotopic model, human tumor material is introduced at the site of the primary tumor source. Accordingly, the xenograft grows in the tissue of origin of the primary tumor and might more faithfully mimic human carcinogenesis and later metastatic events. Given that skillful surgical intervention is often needed for tumor implementation, the number of available surgically manipulated mice will be limited. Disease onset and dissemination patterns may also vary not only between mice and humans but also among mice, thereby further complicating execution of individual tests and validation of statistical data. In contrast to patients, in whom primaries are as a rule surgically removed and morbidity is caused by metastatic disease, mice succumb to primary lesions well before disease spread has occurred. Thus, additional surgical intervention is frequently required to excise the primary lesion, further complicating the implementation of orthotopic models.

Despite the wide availability of human cell lines expressing a certain target molecule, naïve cell lines are also being genetically manipulated to express molecular targets of interest. In a similar approach, they can be programmed to express a foreign label protein with the aim of monitoring tumor induction and propagation in the host. Manipulations of the host genome may also result in spontaneous carcinogenesis in the immunocompetent syngeneic host. Transgenic mice may be engineered by exchange of the endogenous sequence of a gene to more closely mimic human carcinogenesis (knock-in mice); alternatively, they may be genetically manipulated by disruption of a gene to suppress its function (knock-out mice). Today, transgenic mice may develop cancers in a diversity of organs with an inherently controlled progression closely resembling human carcinogenesis. Tumors develop spontaneously and have histologic similarities to human tumors with which they share many molecular and genetic traits. However, the use of genetically engineered mice has been limited in translational research of new therapeutics not only as a result of their cost and limited availability but also because of logistic and practical issues. For most healthy animals, including humans, cancer is a disease of old age, occurring after a lifetime of DNA damage, which results in the loss of replication control in some cells. Testing chemicals for cancer induction potency is challenged by the tendency for many test species to develop spontaneous tumors late in life, as well as by the number of animals surviving long enough for tumor induction to be observed. Because the period of cancer onset and subsequent metastatic spread (known as the risk period) can greatly vary between members of the same group of genetically engineered mice, it becomes practically challenging to conduct a statistically tight evaluation study.

## HUMANS VERSUS MICE: CONSEQUENCES OF SIZE DIFFERENCE

An important difference between species such as mice and humans in preclinical evaluation studies is the most obvious one, namely their size. The small size of the mouse has important implications for imaging and radionuclide therapy studies, including limitations on the maximum volume to be injected or the maximum volume of blood samples to be taken. For most species, the blood volume in milliliters is approximately 6%–8% of the body weight in grams. In general, without fluid replacement approximately 10% of the total blood volume can be safely removed at one time, whereas with fluid replacement up to 15% can be removed. So, without fluid replacement, up to 0.2 mL of blood can be taken from a 25-g mouse; with fluid replacement, up to 0.3 mL. With regard to the maximum volume that may be injected, an injection volume of 0.2 mL can be safely given to an adult mouse. Because of the small maximum volume to be injected, the specific activity of the radiopharmaceuticals should be high, especially when the processes to be studied have low capacity and can be saturated readily, such as when receptor binding is involved. This might present a problem when non-carrier-added levels are not possible. Also, because sensitivity remains a limiting factor in small-animal imaging studies, radiation dose in small animals may be high, especially when scans are being repeated over time. For imaging, another apparent implication of the small size of mice in comparison to humans is the need for a much higher resolution of the imaging systems to be used than in the clinical situation. Nevertheless, mice lend themselves well to multimodality imaging studies using not only radionuclides but also ultrasound, optical imaging, and anatomic imaging.

When comparisons are made between species of different sizes for a variety of different physiologic parameters, a wide variation across body size is seen. For example, the heartbeat rate of a mouse is about 600 beats per minute, compared with 80 per minute for a human. As a result of the faster rate, various other physiologic processes are faster in small animals than in humans. Thus, the reason that small animals can generally tolerate larger doses (in mg/kg of body weight) of a pharmaceutical is that they are able to clear most chemicals from their bodies much more quickly than humans. The longer biologic half-lives of chemicals in humans, compared with small animals, means that a given dose will lead to higher concentrations of the chemical in the tissues of a human than in the tissues of a small animal. It is nevertheless generally believed that pharmacokinetic data can be extrapolated to humans reasonably well, using the appropriate pharmacokinetic principles. This belief has been formalized in the concept of allometric scaling, which states that anatomic, physiologic, and biochemical variables in mammals (such as tissue volumes, blood flow, and process rates) can be scaled across species as a power function of body weight (5,10,11).

## ANIMAL HANDLING AND ANESTHESIA

Several recent studies have demonstrated the impact of animal handling and various anesthetic agents on the results of animal studies. To maintain a consistent physiology between animals and across studies, the preparation of the animal must be uniform and the experimental imaging or therapeutic protocol identical. This requirement involves maintaining body temperature and monitoring vital signs throughout the study. Many manufacturers of commercial imaging equipment now recognize the importance of physiologic monitoring and control and include these systems as part of the imaging equipment. However, important confounding factors are introduced in small-animal imaging that could make translation to humans more difficult. One of the most significant factors is the use of anesthesia in animals. Anesthetics are known to alter animal physiology dramatically, causing changes in respiration, heart rate, blood pressure, and temperature. In addition, the various types of anesthetics cause different effects that are dose-dependent as well (11,12). As a consequence, any interpretation of imaging results in small animals must include an analysis of the effects of the anesthetic before the interpretation can be translated to humans.

## ANIMAL RESEARCH QUALITY

In small (and often also in larger) research groups, the design of animal studies, the experimental execution, and the evaluation of the data are under the purview of one, nonmasked, person. Several studies elucidated that certain weaknesses in many animal studies, including this lack of masking, limit their translational value to human application.

Bebarta et al. reviewed 290 animal experiments presented at emergency medicine meetings (13). When the data from abstracts describing animal studies that used both randomization and masking were compared with data from studies that used neither, the latter studies were 5 times more likely to report a difference between study groups than studies that used these methods, indicating that also in animal studies it is important to include masking and randomization in the experimental design.

Perel et al. compared treatment effects in animal models with those obtained in human clinical trials for 6 interventions that showed definitive proof of benefit or harm in humans (14). They used systematic reviews of human and animal trials to analyze the effects of 6 (not nuclear medicine-related) drugs for conditions such as head injury, stroke, and osteoporosis. Overall, there was only a 50% concordance between animal studies and clinical studies. Lack of concordance between animal experiments and clinical trials appeared again to be due to lack of masking, lack of randomization, or failure of the animal models to adequately represent human disease. This failure of the models to represent human disease can be caused by several factors. Test animals are often young, rarely have comorbidities, and are not exposed to the full range of

interventions that humans often receive. In addition, the timing, route, and formulation of the intervention in animal studies may introduce translational problems. Optimism bias may also play a role: investigators may select positive animal data but ignore equally valid but negative work when planning clinical trials.

We have not systematically reviewed the translational success of radiopharmaceuticals for imaging or therapy in nuclear medicine. Nevertheless, also in our field more uniform experimental design and reporting requirements might improve the quality of animal research, requiring agreement and cooperation between investigators, editors, and funders of basic scientific work.

## CONCLUSION

Animal studies are required before human administration of new medicines, including radiopharmaceuticals. Animal studies can be of great value, even though results in animals are sometimes not fully applicable to humans because of inherent biologic differences between the species. Decisions on the choice of a relevant experimental model and the design, execution, and evaluation of the experiments have to be made carefully. It is good to remain critical and cautious about the applicability of animal data to the clinical domain.

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Because of space limitations, we were unable to cite as many primary references as we would wish; instead, we decided to steer the reader toward some excellent reviews.

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