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Review Article

Lost in translation: animal models and clinical trials in cancer treatment

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Abstract: Due to practical and ethical concerns associated with human experimentation, animal models have been essential in cancer research. However, the average rate of successful translation from animal models to clinical cancer trials is less than 8%. Animal models are limited in their ability to mimic the extremely complex process of human carcinogenesis, physiology and progression. Therefore the safety and efficacy identified in animal studies is generally not translated to human trials. Animal models can serve as an important source of *in vivo* information, but alternative translational approaches have emerged that may eventually replace the link between *in vitro* studies and clinical applications. This review summarizes the current state of animal model translation to clinical practice, and offers some explanations for the general lack of success in this process. In addition, some alternative strategies to the classic *in vivo* approach are discussed.

Keywords: Animal models, translational studies, clinical trials, cancer, review

Introduction

Prior to embarking on cancer drug trials, pharmaceutical companies and independent investigators conduct extensive pre-clinical studies. *In vitro* (test tube or cell culture) and *in vivo* (animal experiments) studies examine preliminary efficacy, toxicity and pharmacokinetics. Early *in vivo* testing specifically aims to demonstrate safety, which assists investigators to determine whether a candidate drug has scientific merit to justify further development. Both the Food and Drug Administration (FDA) and Health Canada require that animal tests be conducted before humans are exposed to a new molecular entity [1, 2].

The ultimate goal of cancer researchers is to translate scientific findings into practical clinical applications. Experimental discoveries are thought to begin at "the bench" with basic research, progress through pre-clinical animal studies, then show therapeutic efficacy in human clinical trials. Although animal models continue to play a large role in the evaluation of efficacy and safety of new cancer interventions, genetic, molecular, and physiological limitations often hinder their utility. Despite successful pre-clinical testing, 85% of early clinical trials for novel drugs fail; of those that survive through to phase III, only half become approved for clinical use [3]. The largest proportion of these failures occurs in trials for cancer drugs [4]. Furthermore, fewer than one in five cancer clinical trials find their way to the peer-reviewed literature, generally due to negative findings [5]. Although logistical and study design issues are often identified as the root cause of clinical trial failures, most futilities in fact originate from molecular mechanisms of the drug(s) tested [6].

The overall result is that promising pre-clinical animal studies that require extensive resources both in time and money rarely translate into successful treatments. This review provides a critical evaluation of pre-clinical animal models and their role in translation to clinical practice for cancer patients.

Limitations of animal models in cancer research

Animal models have not been validated as a necessary step in biomedical research in the

scientific literature [7]. Instead, there is a growing awareness of the limitations of animal research and its inability to make reliable predictions for human clinical trials [8]. Indeed, animal studies seem to overestimate by about 30% the likelihood that a treatment will be effective because negative results are often unpublished [9]. Similarly, little more than a third of highly cited animal research is tested later in human trials [10]. Of the one-third that enter into clinical trials, as little as 8% of drugs pass Phase I successfully [11].

The major pre-clinical tools for new-agent screening prior to clinical testing are experimental tumors grown in rodents. Although mice are most commonly used, they are actually poor models for the majority of human diseases [12]. Crucial genetic, molecular, immunologic and cellular differences between humans and mice prevent animal models from serving as effective means to seek for a cancer cure [13]. Among 4,000+ genes in humans and mice, researchers found that transcription factor binding sites differed between the species in 41% to 89% of cases [14]. In many cases, mouse models serve to replicate specific processes or sets of processes within a disease but not the whole spectrum of physiological changes that occur in humans in the disease setting [15].

The failure to translate from animals to humans is likely due in part to poor methodology and failure of the models to accurately mimic the human disease condition. The core of the problem may be rooted in the animal modeling itself. Unlike in human clinical trials, no best-practice standards exist for animal testing [14]. Moreover, the laboratory environment can have a significant effect on experimental results, as stress is a common factor in caged mice [16]. It has been recommended that therapeutic agents should not only be evaluated in rodents, but also in higher animal species, and that randomization and outcomes assessor blinding should be performed. In addition, experiments should be designed in both genders and in different age groups of animals and all data, both positive and negative, should be published [3].

Notable examples of failed clinical cancer trials initiated due to successful animal models

A well-known example of a successful animal model that did not translate into clinical trials is the TGN1412 trial [17]. The drug TGN1412, developed by the company TeGenero, was described as an immunomodulatory humanized agonistic anti-CD28 monoclonal antibody developed for the treatment of immunological diseases such as multiple sclerosis, rheumatoid arthritis and certain cancers. Before conducting human trials, TGN1412 was tested on different animals including mice, to ensure safety and efficacy in preclinical animal models [17]. These toxicity studies demonstrated that doses hundred times higher than that administered to humans did not induce any toxic reactions. In the first human clinical trials of TGN1412, the drug caused catastrophic systemic organ failure in patients, despite being administered at a sub-clinical dose that was 500 times lower than the dose found safe in animal studies [18].

In a recent report, a Phase II randomized clinical trial of the Hedgehog pathway antagonist IPI-926 (saridegib) in patients with advanced chondrosarcoma was stopped early for futility [19]. The Hedgehog pathway is dysregulated in a variety of solid tumors and provides key growth and survival signals to tumor cells. Mutations resulting in constitutive Hedgehog signaling are causal in cartilage tumors such as chondrosarcoma [20]. The Phase II clinical trial for IPI-926 translated from a successful animal model of IPI-926 on a malignant solid brain tumor [21]. IPI-926 treated mice with the advanced brain tumors gained a fivefold increase in survival [21]. However, IPI-926 showed no effect compared to placebo in the human trial [19]. Therefore even a targeted molecular approach did not result in clinical efficacy despite remarkable success in mice.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent proteinases involved in the degradation and remodeling of extracellular matrix proteins and are associated with the tumorigenic process. MMPs promote tumor invasion and metastasis, regulating host defense mechanisms and normal cell function [22]. Cancer and arthritis were once regarded as the prime indications for the use of MMP inhibitors (MMPIs) and results from multiple animal studies indeed indicated that MMP inhibition would be an effective therapeutic approach in the management of cancer and other diseases [15]. However, multiple failed clinical trials in humans have had the effect of

seriously reducing interest in MMP inhibition as a valid therapeutic option [22].

Among the more-than 16 MMPIs that progressed to clinical testing, only Periostat (doxycycline hyclate, a nonspecific MMPI) has been approved for clinical use in periodontal disease [15]. The serious safety problems in clinical trials have been attributed to poor selectivity of the MMPIs, poor target validation for the targeted therapy and poorly defined predictive preclinical animal models for safety and efficacy [23]. The failure and indeed resulting damage of all anti-MMP drugs in clinical trials indicated that MMPs as a class have useful functions in normal tissue, and therefore inhibition would result in toxicities in the human host not identified in the animal models in which they were tested.

Therapeutic cancer vaccines are becoming increasingly popular in the approach to cancer treatment. The concept of stimulating the body's immune system to fight tumors, representing an alternative approach to the use of traditional cytotoxic cancer therapies, is indeed compelling [24]. A typical therapeutic vaccine against cancer contains a cancer-specific peptide, or protein fragment, that is injected under the skin of either the tested animals or humans. It is assumed that the immune system would recognize the peptide as something to be attacked and boosts the population of cancer-fighting T-cells in the bloodstream [25]. These vaccines must first be tested in animals to confirm efficacy prior to entering into human clinical trials [26]. In the particular case of cancer, preclinical animal models have provided new knowledge regarding vaccine-induced immune responses and the central importance of T cell-mediated cellular responses in cancer treatment [25].

Although therapeutic cancer vaccines have been effective in initiating the immune response in animal models, they have produced mixed results in human clinical trials. In a recent review article, it was reported that out of 23 Phase II/III clinical trials testing 17 distinct therapeutic anticancer vaccines, 18 of these studies had failed [27]. Some examples are Merck's Stimuvax (failed a phase III trial on non-small cell lung cancer) [28], GlaxoSmithKline's MAGE-A3 (failed a phase III melanoma trial) [29], Vical's Allovectin (failed a phase III metastatic melanoma trial) [30], and KAEL-GemVax's TeloVac (failed a phase III pancreatic cancer trial) [31]. It has been postulated that most of the cancer vaccine trials have failed due to elevated levels of circulating immunosuppressive cytokines and various immunological checkpoints in humans that may not be present in rodents [25].

Critical re-evaluation of animal models and alternative strategies

Despite the general lack of success in translating animal models to clinical studies, animals are still prevalently used in laboratories all over the world to test the safety, toxicity and effectiveness of drugs [32]. Animal models have been essential in cancer research for obvious practical and ethical concerns associated with human experimentation. Animal research is similar to *in vitro* assays, epidemiological investigations, and computer simulations. All attempt to derive probabilistic knowledge in one context that will generalize to humans. All are forms of modeling that will map onto the whole population with less than perfect precision and predict with even less precision the fate of any individual. Notwithstanding, these methods risk missing some important knowledge, or risk finding knowledge that doesn't hold up in the clinical setting even to a point that is actually harmful once widely deployed.

Ultimately, we come into the question as to whether we should spare resources and bypass animal models to evaluate therapy in humans directly. In the last decade, the FDA and the European Medicines Agency introduced guidelines for testing very small 'micro-doses' of drugs in humans [33]. These are concentrations less than a one-hundredth of the therapeutic dose. Because the concentrations are so low, the drugs can be tested in a small number of patients without the level of safety data normally required before a phase I study. These early 'phase 0' studies collect human data quickly by showing how the drug is distributed and metabolized in the body, and whether it hits the right molecular target. Approximately one-quarter of the molecules entering clinical trials fail due to pharmacological issues such as lack of absorption or penetration into the target organ [33]. With a direct test in humans, pharmaceuticals can determine earlier whether the drug is worth investing both time and

money into clinical research. Phase 0 trials may be small in scope, but they require very sensitive tests to detect the minute quantities of the drug in the body and possibly its mechanism of action.

Aside from phase 0 studies, a wide range of alternatives to animal-based preclinical research has emerged. These include epidemiological studies, autopsies, *in vitro* studies, *in silico* computer modelling, "human organs on a chip" - creating living systems on chips by mimicking a micro- biological environment with cells of a certain organ implanted onto silicon and plastic chips [34], and "microfluidic chips" - automation of over a hundred cell cultures or other experiments on a tiny rubbery silicone integrated circuit with miniscule plumbing [35]. The National Institutes of Health of the United States suspended all new grants for biomedical and behavioural research on chimpanzees after an expert committee concluded that such research was unnecessary [36]. Furthermore, the US National Research Council recommends that animal model based tests be replaced as soon as possible with *in vitro* human cell-based assays, *in silico* models, and an increased emphasis on epidemiology [37].

In summary, animal models have been the basic translational model in the preclinical setting in elucidating key biochemical and physiologic processes of cancer onset and propagation in a living organism. Experimental tumors raised in animals, particularly in rodents, constitute the major preclinical tool of evaluating novel diagnostic and therapeutic anticancer drugs screening before clinical testing. The power of the animal models to predict clinical efficacy is a matter of dispute due to weaknesses in faithfully mirroring the extremely complex process of human carcinogenesis. The vast majority of agents that are found to be successful in animal models do not pan out in human trials. Differences in physiology, as well as variations in the homology of molecular targets between mice and humans, may lead to translational limitations. Even though animal models still remain a unique source of *in vivo* information, other emerging translational alternatives may eventually replace the link between *in vitro* studies and clinical applications.

Disclosure of conflict of interest

None.

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