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Animal models of disease: Pre-clinical animal models of cancer and their applications and utility in drug discovery

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ABSTRACT

Preclinical models of human cancers are indispensable in the drug discovery and development process for new cancer drugs, small molecules and biologics. They are however imperfect facsimiles of human cancers given the genetic and epigenetic heterogeneity of the latter and the multiplicity of dysregulated survival and growth-regulatory pathways that characterize this spectrum of diseases. This review discusses pre-clinical tumor models – traditional ectopic xenografts, orthotopic xenografts, genetically engineered tumor models, primary human tumorgrafts, and various multi-stage carcinogen-induced tumor models – their advantages, limitations, physiological and pathological relevance. Collectively, these animal models represent a portfolio of test systems that should be utilized at specific stages in the drug discovery process in a pragmatic and hierarchical manner of increasing complexity, physiological relevance, and clinical predictability of the human response. Additionally, evaluating the efficacy of novel therapeutic agents emerging from drug discovery programs in a variety of pre-clinical models can better mimic the heterogeneity of human cancers and also aid in establishing dose levels, dose regimens and drug combinations for use in clinical trials. Nonetheless, despite the sophistication and physiological relevance of these human cancer models (e.g., genetically engineered tumor models and primary human tumografts), the ultimate proof of concept for efficacy and safety of novel oncology therapeutics lies in humans. The judicious interpretation and extrapolation of data derived from these models to humans, and a correspondingly greater emphasis placed on translational medical research in early stage clinical trials, are essential to improve on the current clinical attrition rates for novel oncology therapeutic agents.

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1. Introduction

In considering the various types of animal cancer models described in this overview, it is imperative to realize that they are imperfect representations of the complex, diverse and multifaceted spectrum of genetic diseases that encompass human cancer. Thus cancer is not a single disease state, as a simple reductionist view might suggest, but by its very nature exhibits considerable intra- and inter-tumor heterogeneity both genotypically and phenotypically that are both dynamic and variable in nature, with most cancers utilizing multiple and redundant dysregulated survival and growth-regulatory pathways in the course of their

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adaptive evolution [1–4]. Over the past decade, seminal studies employing global genomic analyses and deep sequencing techniques both across cancer genomes and in specific types of cancers have revealed the molecular basis for this heterogeneity and the evolutionary diversity of human cancers. These analyses have demonstrated the considerable incidence, patterns, and variety of genetic alterations (somatic mutations, gene amplifications, deletions) and epigenetic alterations in human cancers and their temporal and spatial nature in relation to tumor development [5–11]. These complex and adaptive genetic and epigenetic profiles manifest as distinct differences in metabolic, proliferative, developmental, and epidemiological profiles impacting both tumor growth and survival and the responsiveness (or lack thereof) of tumors to a multiplicity of mechanistically distinct therapeutic agents [1,12,13]. This genetic and epigenetic complexity and diversity and the resulting phenotypic heterogeneity and resiliency that are characteristic hallmarks of human cancers must be considered in the interpretation and extrapolation of experimental data generated in preclinical models of human cancer and their potential relevance in evaluating new therapeutic agents for the cancer patient.

Animal models of human cancer and the in vivo biological, pharmacodynamic/pharmacokinetic (PD/PK) and pharmacological information they can provide remain critical components in: (i) understanding the pathophysiology of cancer including new target identification; (ii) identifying novel therapeutic agents; (iii) exploring the utility of novel therapeutics in combination with, or adjunct to, established chemo- and radio-therapeutic regimens and approved targeted therapeutic agents; and (iv) in studying mechanisms of intrinsic and acquired resistance to cytotoxic and targeted therapies. Despite differences in the types of models discussed below, in general, tumor development is more rapid and homogeneous in murine models as compared to the heterogeneity of human cancers discussed above, while offering considerable practical benefits for drug discovery, development, translational biology and biomarker assessment of anti-cancer therapeutic agents [14–19]. These models also have limitations, like the majority of animal models irrespective of the therapeutic area – in modeling the human disorder. When interpreted appropriately to address specific experimental questions and end-points, these models have an appropriate and critical niche in oncology drug discovery.

The practical need for facile, cost-efficient, and pharmacologically relevant preclinical animal models of human cancer is clear: drug discovery and development is a high-cost and high-risk endeavor requiring an average expenditure in excess of $1 bn [20,21] and 10–12 years from a laboratory concept to FDA approval. Reported attrition rates for oncology drugs are historically inconsistent often due to the manner in which clinical data have been generated, the size and duration of clinical trials for cytotoxic versus targeted therapeutic agents, and the considerable differences in the breadth of resources and infrastructure supporting the oncology pipelines of larger pharmaceutical companies versus smaller biotech companies [22]. For example, earlier assessments by Kola and Landis [23] reported only a 1 in 20 success rate (95% attrition) for oncology drugs, while more recent analyses [24,25] reported an attrition rate of approximately 70% for oncology drugs. Nonetheless, despite these high attrition rates and the relatively long development times for oncology drugs (8.1 years), the number of approved oncology drugs has increased approximately 5-fold since the early 1980s, with the number of approvals similar to that of cardiovascular drugs in the past decade [24,25]. Of note, the highest probability of attrition for all oncology drugs remains during Phases II and III of clinical development [22–25]. Despite the successes in oncology drug approvals, these high attrition rates, particularly in the more costly Phase II and III clinical stages are often seen as an indictment on the limited clinical relevance and predictive power of available pre-clinical cancer models. While such limitations must be recognized in the context of the particular strengths, weaknesses, applications and predictive power of various pre-clinical cancer models, the use of impractical and often outdated, 'non-adaptive' clinical trial designs for targeted therapeutic agents have also been seen as a major problem contributing to the high attrition rates and long development times for oncology drugs [26–28].

Animal models of cancer encompass a wide spectrum and include: (i) *ectopic xenografts* (subcutaneous (sc), intraperitoneal (ip), intravenous (iv), intramuscular (im)) of tumor-derived cell lines or tissue explants implanted into syngeneic or immune-compromised rodent hosts; (ii) *orthotopic models* in which explants of tumor tissues or established tumor lines are implanted within the proper organ or tissue, thus recapitulating the intricacies and cell-cell interactions of the local microenvironment in which a primary tumor grows and from which it invades and disseminates; (iii) *germ-line transgenic and conditional transgenic models* (GEMMs) in which the expression patterns of specific oncogenes or tumor suppressor genes can be regulated systemically or in a tissue- and temporal-specific manner, respectively; (iv) *primary human tumorgrafts* maintaining the genotypic and phenotypic profile of a primary tumor from which they are derived; and (v) *various carcinogen-induced models* that recapitulate the time-dependent and multistage progression of tumor pathogenesis in response to environmental carcinogens and tumor-promoting agents. The strengths, weakness, applicability and predictability to human disease of these classes of pre-clinical models in the context of drug discovery are discussed in this review and highlighted in Table 1.

Excellent and comprehensive reviews [15,17,19,29–32] are available on the history and development of these specific types of pre-clinical tumor models in rodents and their applications, advantages, and limitations in oncology drug discovery and development.

2. Classes of pre-clinical cancer models: applications and clinical predictability in oncology drug discovery and development paradigms

2.1. Ectopic tumor xenografts using established human and murine tumor cell lines in immune-deficient and immune-competent mice

In the early 1970s it was demonstrated that human tumor tissues could be successfully propagated in athymic *nu/nu* mice, leading to ectopic tumor xenografts becoming a valuable and generally accepted biological approach to the study of cancer biology and therapeutics [14,15,17,18]. Ectopic tumor xenograft models employing sc, ip, or im implantation of tumor cell lines or tissue explants into syngeneic (genetically identical) and immunocompromised rodents are invaluable in evaluating reproducibly and quantitatively the pharmacological consequences (tumor PD/PK relationships and anti-tumor efficacy) of modulating a specific molecular target or pathway for the in vivo screening and facile assessment of new chemical entities (NCEs) emerging from drug discovery paradigms. In this regard, they are useful in assessing both anti-tumor efficacy and overall tolerability in vivo in the early screening of NCEs due to their reproducibility, modest throughput, cost- and time-effectiveness, and feasibility across a variety of tumor cell types. Similarly, the use of tumor cell line based ectopic xenograft models affords a facile and effective means of obtaining important translational biology data on NCE emerging from drug discovery efforts. For example, these types of models are useful for evaluating dose response and plasma and tissue exposure and tumor PD in relation to efficacy; in the evaluation of alternative dosing schedules and frequencies and