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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

Table 1
Characteristics and applications of classes of animal models of cancer and their utility in oncology drug discovery.

Type of cancer model	Species	Primary features	Utility	Strengths and advantages	Weaknesses and caveats	Clinical predictability
Sc, iv, ip ectopic xenograft models with established tumor lines	Immuno-competent and immuno-deficient rodents	- Ectopic implantation of cell lines or tissue fragments - Non-physiologic growth location	- Facile and rapid pharmacological screening of NCEs throughout discovery screens - Tumor PK/PD relationships - Anti-tumor efficacy	- Reproducible - Cost and time effective tumor measurements - Applicable to many tumor cell types - Assess influences of immune surveillance and evasion (immuno-competent hosts)	- Limited histological and phenotypic similarities to primary cancers - Loss of tumor heterogeneity - Low metastatic rates - Lack of native tumor microenvironment	- Limited to poor predictiveness for most cancers with noted exceptions (e.g., oncogene driver mutations)
Orthotopic tumor models with established tumor lines	Immuno-competent and immuno-deficient rodents	- Implantation into organ of origin - Reconstitutes organ micro-environment - Local and metastatic spread	- Mid-later stage discovery screening - Assess survival endpoints - Evaluate effects on primary tumor growth in proper microenvironment - Assess influence on local and metastatic tumor spread	- High metastatic rates - Correct tumor microenvironment - Assessment of tumor-stromal interactions - Assess anti-tumor efficacy-of primary and metastases - Assess influence on immune surveillance and evasion (immuno-competent hosts)	- More time and labor intensive - In vitro artificial selection of cell lines - Histological dissimilarities with human tumors - Loss of tumor heterogeneity - Imaging modalities needed for in situ assessment of tumor development	- Limited–moderate predictiveness - Useful to model clinical course of metastatic disease
Transgenic and GEMM tumor models	Immuno-competent mice	- Spontaneous and autochthonous development of tumors in appropriate micro-environment in situ	- Mid-later stage discovery screening - Evaluate effects on immune surveillance, invasion and metastasis, and tumor angiogenesis	- Phenotypic, histological and genetic similarities to primary cancers - Models human cancer under more physiological conditions - Assess influences on tumor immune surveillance and evasion	- Asynchronous development of tumors - Out bred mice with non-uniform genetic backgrounds - More time and labor intensive - Metastatic patterns may not mimic human cancers - Lack of tissue-specific promoters - Imaging modalities needed for in situ assessment of tumor development	- Predictive in therapeutic responsiveness and in development of therapeutic resistance
Primary human tumorgraft models	Immuno-deficient mice	- Direct implantation/ propagation of freshly excised human tumors	- Mid-later stage discovery screening - Evaluate NCE effects on original human tumor ectopically or orthotopically	- Preserves and stabilizes genetic, histological, and phenotypic features primary tumor - Maintains stromal and stem cell components of primary tumor - Facilitates biomarker assessment - Potential to metastasize - Ease of tumor measurements	- Access to freshly excised human tumors - High front-end costs and labor-intensive preparation - Limited engraftment rates and long latency for tumor development	- Highly predictive and physiological; used for "clinical trials in a mouse"
Carcinogen-promoter-induced multi-stage tumor models	Immuno-competent rodents (mice, rats, hamsters)	- Time-dependent, multi-stage progression of tumor development in response to carcinogens and tumor-promoting agents - Etiologically relevant environmental carcinogens induce tumors in organ-specific manner	- Later stage discovery screens - Determine most sensitive stages of tumor development for chemo-preventive and or therapeutic intervention	- Histological, biochemical and phenotypic similarities to human cancers - Mimic stage-specific developmental sequelae of cancers - Reproducible and high penetrance of organ-specific lesions - Metastasize to specific sites - Assess influences on tumor immune surveillance/evasion	- Long time frames (5–50 weeks) for tumor development - High costs in animal maintenance/care - Repeated use of carcinogenic agents - Out bred mice with non-uniform genetic backgrounds and varied sensitivity to carcinogens - Imaging modalities needed for in situ assessment of tumor development (non skin lesions)	- Predictive of developmental sequelae of human cancers - Favorable predictive responsiveness to therapeutic and chemo-preventive agents

the impact of discontinuation of drug administration on the duration and magnitude of anti-tumor efficacy; and in exploring the efficacy and tolerability of various combination dosing regimens of NCE with standard of care cytotoxic agents, radiotherapy and targeted therapeutic agents for a specific cancer.

Despite these utilities, tumor cell line based ectopic xenograft models possess limited pathophysiological relevance and clinical predictability [31,33–35] given their histological features, genetic profiles, loss of inter- and intra-tumor heterogeneity, non-physiological growth location in the host, and the absence of critical stromal and micro environmental spatial and paracrine interactions with host non-cancerous cells and tissues, including endothelial cells, inflammatory cells and tumor-associated fibroblasts, and matrix proteins [13,33,36–38]. These limitations and caveats in the use of tumor cell line-based ectopic xenograft models lie in the nature and inherent biological properties of the tumor cell lines on which they are based [37,38]. Over the past 25 years, government and academic institutions have established panels of well-characterized human tumor cell lines ranging from the NCI-60 cell line panel [39] to the Cancer Cell Line Encyclopedia of 1200 cell lines [40] as repositories that could be used to model and reproduce the variability of response of specific types of tumors to a variety of chemotherapeutic agents (the original intent of the NCI-60 panel), as well as capture the extent and variety of genetic and epigenetic heterogeneity in specific cancers impacting their responsiveness to genetically based targeted therapies. Although these panels of cell lines generated a wealth of information in understanding and studying cancer biology and the development of new therapeutic agents, they have several inherent shortcomings that must be considered in the context of their use for in vitro and in vivo studies in oncology drug discovery. Most tumor cell lines have been maintained for decades in enriched growth media, grown as monolayer cultures (not in 3-dimensional matrices) and under non-physiological oxygen tensions, resulting in the artificial selection of tumor cell lines to generate primarily undifferentiated tumor cell clones with high proliferative potential that are not generally representative of the genetic and epigenetic heterogeneity of the original primary tumor [15,31,37,38,41]. Prolonged culture of these cell lines in vitro under such non-physiological conditions impacts their genetic fidelity, inducing additional genetic alterations, gene copy number variations, and variants in transcriptional profiles compared to the primary tumors from which they were derived. Several studies have demonstrated that although critical genetic "driver" mutations [6] are often retained in tumor cell lines (as discussed further below), the loss of genetic fidelity results in tumor cells lines that more closely resemble each other regardless of tissue origin than the primary tumor from which they arose [37,38,41]. These more homogeneous and in vitro selected tumor cell lines can often afford rapid and predictable ectopic xenograft growth in vivo, but result in tumor models with limited phenotypic, histological, and genotypic similarities to most primary human cancers. Moreover, the metastatic rates from sc or im tumor xenografts in murine hosts have been low or nonexistent [13,15,31,33] due in large measure to the same deficiencies noted above, particularly the limited ability of ectopic xenograft models to afford critical tumor stromal and tumor microenvironmental cues and the essential collective growth factor, chemokine, and cytokine milieu in situ. The complex spatial and temporal integration of these dynamic interactions exert positive and negative influences on tumor growth, survival, differentiation, epithelial-mesenchymal transitions (EMT), angiogenesis, and immune surveillance-collectively impacting primary tumor metastatic dissemination to distant sites [1,13,42]. Therapeutic modulation of these types of critical tumor-micro environmental interactions and their impact on tumor metastasis, vascularization, and the emergence of acquired tumor resistance have been the focus of many targeted therapeutic agents in oncology, but can be only marginally evaluated using traditional ectopic tumor cell line derived xenograft models [13,18,31,38,42]. Consequently, for the vast majority of human cancers (non-small cell lung (NSCL) cancer being a notable exception) reliance upon sc and systemic tumor xenograft models alone as a measure of anti-tumor efficacy or to study therapeutic impact on metastasis, vascularization, and emergence of tumor resistance, has limited therapeutic utility and predictability for patient responses and clinical outcomes, particularly for targeted therapeutics versus traditional cytotoxic anti-cancer agents. These assertions are supported by several salient retrospective studies on the limited predictive value of traditional ectopic tumor cell line derived xenograft models in relation to clinical responsiveness to therapeutic agents [31,34,35,37].

Notable exceptions to these observed limitations in the clinical predictability of traditional ectopic tumor xenografts are human tumors which are highly dependent upon specific oncogenic "driver mutations" for their growth and survival [1,5,6]. In many of these instances, the responses obtained in traditional ectopic xenograft models derived from tumor cell lines carrying the "driver" genetic mutations of interest have been representative of the clinical responses observed in cancer patients treated with targeted therapies directed against these mutated "driver" oncoproteins, at least initially prior to the almost inevitable development of acquired therapeutic resistance in these patients. There are notable examples to illustrate the translational relevance of specific types of ectopic xenograft models. Among these examples include the marked responsiveness of NSCL cancer ectopic xenografts harboring activating genetic alterations (mutations, translocations, amplifications) in the epidermal growth factor receptor (EGFR) to first-generation kinase inhibitors of EGFR such as gefitinib and erlotinib [43,44], and the similarly robust anti-tumor efficacy (tumor regressions) observed in erlotinib-resistant NSCL ectopic xenografts bearing acquired T790M resistance mutations in the EGFR kinase when tumor bearing animals were treated with second-generation, irreversible inhibitors of EGFR and HER2 [45,46]. Similarly, ectopic xenograft models of malignant melanoma bearing activating mutations in the B-Raf oncogene respond to B-Raf inhibitors such as vemurafenib and dabrafenib with marked tumor growth inhibition and tumor regressions [47–49] similar to that observed in subsets of malignant melanoma patients bearing the mutated B-Raf genotype and treated with these targeted therapies [50–52]. In contrast to these observations, in B-Raf mutated colorectal cancer, there is a marked discordance between the significant anti-tumor efficacy observed in B-Raf mutated ectopic xenograft model of colorectal cancer [53] and the marginal clinical response rates observed in B-Raf mutated colorectal cancer patient sub-populations. These clinical observations are due in large measure to compensatory activation of EGFR and reactivation of MAPK survival signaling pathways in patients' tumors following treatment with B-Raf inhibitors [54,55].

The ALK oncogene is constitutively activated in subsets of anaplastic large-cell lymphoma (ALCL) and subsets of NSCL cancer through specific chromosomal translocations and generation of tumor-specific oncogenic fusion proteins [56,57]. The significant tumor growth inhibition and high incidence of tumor regressions achieved in pre-clinical ectopic tumor xenograft models of ALCL [58] and NSCL [59–61] are reflective of the dramatic objective clinical responses and improvements in patient survival in patients with ALK positive tumors receiving targeted ALK kinase inhibitor therapies [56,57]. In these instances, traditional ectopic xenografts of the appropriate tumor type and genotype signature have been predictive in some, but not all, cancers of the initial objective tumor responses and survival benefits of targeted therapeutics to these mutated oncoproteins in cancer patients.

2.2. Orthotopic tumor models using established human and murine tumor cell lines in immuno-deficient and immuno-competent mice

Orthotopic cancer models in syngeneic and immune-compromised rodents involve the implantation of tumor cells or primary tumor tissue explants into the originating tissue site of the cancer in rodents, resulting in much higher metastatic rates and a pathological phenotype that more closely recapitulates the human clinical course of metastatic disease [13,15,17,62–65]. Compared to the ectopic sc tumor-implantation model, orthotopic tumor implantation more closely simulates the natural environmental milieu of the original tumor, with intact pathological and immunological responses. Stable, reliable and reproducible orthotopic animal models are critical, as they provide an opportunity for studying the mechanisms of pathogenesis as well as malignant progression, i.e., localized invasion and distal metastatic spread of the primary tumor, process important to elucidate in the discovery and development of novel therapeutic agents. Orthotopic implantation combined with subsequent harvesting at metastatic sites can generate cancer cell variants that are clinically relevant to the metastasis process. Thus, in contrast to ectopic sc tumor xenograft models, orthotopic models can more accurately reconstitute an organ microenvironment that dictates the tumor cell phenotype, including the role of tumor-stromal microenvironment interactions on tumor growth and metastasis [13,42,64,65]. The non-malignant cellular components residing in the stromal microenvironment of tumors including inflammatory cells, macrophages fibroblasts, and endothelial cells as well as soluble cytokines, chemokines and cell matrix components and adhesion proteins not only influence the natural history of tumor proliferation, angiogenesis, invasion and metastasis, but are also specifically capable of altering the response (sensitivity versus intrinsic resistance) of tumors to mechanistically distinct anti-tumor and anti-angiogenic therapeutic agents [13,63–65]. Consequently, orthotopic models enable the assessment, chemopreventively or therapeutically, of the effects of specific targeted or cytotoxic agents on primary tumor growth in the appropriate microenvironment, as well as their impact on localized tumor invasion, metastatic spread, and the emergence of acquired therapeutic resistance. These models can also be utilized effectively to evaluate survival endpoints pre-clinically in relation to continuous versus intermittent treatment with novel therapeutic agents or dosing combinations, for example, using Kaplan–Meier analyses [66] of tumor growth inhibition versus tumor regression in the context of survival of tumor-bearing animals.

Although an improvement over ectopic tumor xenografts in this regard, when generated from established tumor cell lines versus primary tissue explants, orthotopic models can suffer some of the same inherent limitations of ectopic xenografts highlighted, e.g., artifactual selection of poorly differentiated, largely homogeneous tumor cell lines selected for high proliferative potential in vivo; tumors with histological and genetic dissimilarities with primary human tumors; and the loss of inter- and intra-tumor heterogeneity that is characteristic of primary human tumors. Orthotopic xenograft models have some additional disadvantages, in that they can be more technically challenging to establish reproducibly in the laboratory and may have highly variable tumor take rates, development times, and animal morbidity associated with orthotopic surgical implantation or injection of tumor cells [13,15,63,64]. Finally, the time and technical labor (organ-specific surgical implantation or injection) needed to generate, maintain, and utilize these more pathologically relevant cancer models relative to ectopic sc or ip implanted tumor models effectively limit their use for the widespread screening and initial evaluation of NCEs during the early drug discovery process. Unlike traditional ectopic sc or ip implanted xenografts in which tumors can be readily measured quantitatively using electronic calipers, quantitative assessment of the impact of therapeutic agents on tumor growth and metastatic progression in orthotopic xenograft models in situ (as well as GEMM – discussed below) relies on the use of imaging modalities, including magnetic resonance imaging, computed and positron emission tomography, Doppler ultrasound, and bioluminescent imaging – all of which have distinct advantages and limitations [13,33,67,68]. The technical and practical considerations of using these various imaging modalities are discussed below in the context of GEMMs.

Despite these considerations, orthotopic models, particularly when utilized in immune-competent syngeneic in bred mouse strains, can have considerable translational impact in the mid-later stages of a drug discovery project and in the pharmacological characterization of optimized leads advancing into development in a syngeneic host with an intact immune system. Examples of commonly used syngeneic orthotopic models in this category include: Lewis lung carcinomas in C57/bl mice, 4T1 mammary carcinoma in Balb/c mice, L210 and P388 acute leukemias in DBA/2 mice, CT-26 colon carcinomas in Balb/c mice, and B16F10 melanomas in C57/bl mice [17,69]. These models address the important consideration of tumor-host compatibility that can arise in using transplanted human tumors in immune-deficient hosts, and can be readily maintained and produced in the laboratory, provided adequate quality controls are maintained and there is access to the specific inbred strains of mice required for these syngeneic mouse models [17,69].

In summary, orthotopic models of human or murine tumors in immune-compromised or syngeneic strains of mice, respectively, are invaluable in understanding the efficacy and tolerability of NCEs administered alone or in combination with established cancer therapies for their impact on primary tumor growth, local invasiveness and distal metastatic dissemination, tumor angiogenesis, the emergence of stromal-associated tumor resistance to therapy, and the survival of animals with metastatic human cancer under more physiologically relevant conditions of tumor growth. In these important respects, orthotopic tumor models in general offer a powerful tool in oncology drug discovery. The question of their correlative predictive power of orthotopic tumor models for clinical response of specific cancers to novel therapies remains unclear. Earlier studies from several investigators described differences between tumor responsiveness and sensitivity in ectopic sc and orthotopic models and reported that orthotopic models are more predictive of clinical response to various cytotoxic oncology therapies, but in some instances, depending upon the nature of the orthotopic model, these may also overestimate clinical responsiveness to specific agents [13,15,17]. Consequently, further in-depth comparative studies are required evaluating both cytotoxic agents as well as targeted therapeutic agents in specific orthotopic versus ectopic tumor models in relation to the clinical responsiveness to these agents before any definitive conclusions can be reached regarding the clinical predictiveness of orthotopic tumor models.

2.3. Transgenic and genetically engineered mouse models (GEMMs) of tumors in immuno-competent mice

Transgenic models, both germ-line and conditionally regulated, are powerful in vivo tools for elucidating complex biological processes linked to the dysregulation of specific molecular or genetic targets (both oncogenes and tumor suppressor genes) in cancers. The availability of specific GEMMs possessing conditional alleles that recapitulate mutations important in specific cancers and can in many instances more faithfully model the full genetic, biochemical, proteomic, histological and phenotypic features of specific human cancers. In this regard, tumors develop

spontaneously and autochthonously in situ in the appropriate tumor-stroma tissue or organ microenvironment of an immunocompetent host [16,31,33,67,70]. This is a major feature that distinguishes GEMMs from traditional ectopic human tumor xenografts, but which they share to some degree with orthotopic xenografts in syngeneic mice, and certainly impacts their predictive and clinical relevance. In this regard, the effects of NCE on the dynamics of the immune response and immune surveillance mechanisms, the localized invasion and systemic metastatic spread of primary tumors, and tumor angiogenesis can be better studied with GEMMs in fully immune-competent host than in ectopic and orthotopic human tumor models in an immune-compromised (nude mouse) or immune-deficient (Scid; Nod-Scid mice) host. The caveat in this regard is that most GEMMs rely on the expression of a single, potent oncogene to drive the tumor, and as a result do not always recapitulate the complete patterns of tumor metastasis seen in cancer patients [32,33,67,70].

Although histologically and genetically similar to human cancers, a major disadvantage to many GEMMs is the asynchronous development of tumors in the transgenic host. These models often have low penetrance and can be heterogeneous with respect to tumor frequency, latency for tumor development, and growth properties – a limitation in a laboratory setting where multiple treatment groups and appropriately powered studies are essential [29,30,67,70]. Similarly, whereas most human ectopic and orthotopic xenografts are grown in inbred immune-compromised murine strains (e.g., athymic nu/nu, and Scid mice), both syngeneic xenografts and transgenic GEMMs are cultivated in out-bred murine strains whose genetic backgrounds may be tumor permissive or tumor suppressive to varying degrees. This non-uniform genetic background of the host can impact outcomes in anti-tumor efficacy studies, contributing to differences in pharmacokinetic, metabolic, and tolerability profiles as well as to heterogeneity in the growth, latency, and progression of tumors. Some of the limitations of asynchronous tumor development in GEMMs can be overcome via the use conditionally regulated GEMMs, allowing control of transgene expression in both a temporal and tissue-specific manner [32,33,67,70,71].

One of the most challenging problems in developing GEMMs of specific cancers is the availability or lack thereof of tissue-specific promoters that conditionally regulate transgene expression exclusively in adult cells of the target tissue in question. The use of tetracycline (Tet)-dependent promoter regulation, *Cre-Lox* or *Flp*-mediated site-specific and spontaneous recombination methods, and somatic cell gene transfer approaches are among the strategies used for the development of high penetrance, clinically relevant, conditionally regulated GEMMs for used in oncology research, with the most clinically relevant and predictable GEMMs being the RIP1-Tag pancreatic islet tumor model, the Eu-Myc Burkitt-like lymphoblastic tumor model, and adenovirus expressing Cre recombinase (*adeno-Cre*) and *Pdx-1-Cre* variants of the *Lox-STOP-Lox-K-Ras2* transgenic mouse used to generate transgenic models of NSCL and pancreatic ductal cancer (PDAC), respectively [29,32,67,70,71].

In the past few years, several developments in GEMMs have been described with the potential for profound impact both in oncology drug discovery and pre-clinical translation biology. For example, several novel GEMMs have been developed for sporadic colorectal cancer based on the delivery via surgical laparotomy of *adeno-Cre* – expressing oncogenic transgenes of interest to the distal colon of *floxed* mice, resulting in the generation of Apc^{CRO}, $Kras^{LSL\text{-}G12D}$, and $p53^{flox/flox}$ GEMM [72,73]. A similar approach was subsequently used to develop a GEMM for B-Raf mutated (V600E) colorectal cancer via administration of adeno-Cre to mice bearing *floxed* Apc and latent Braf alleles [74]. The translational relevance and utility of these models lie in the fact that they mimic sporadic human colorectal cancer based upon somatic alterations of oncogenes directly implicated in the pathogenesis of human colorectal cancer and result in the development of tumors within the colonic microenvironment of an immune-competent host. A practical limitation of these types of GEMMs is that by their nature they are best suited for hypothesis-testing on lead candidate NCEs emerging from drug discovery efforts and in translational biology and biomarker studies on these NCEs, rather than a high throughput in vivo screen to be used early in drug discovery paradigms. Of further translational relevance for oncology drug discovery, however, GEMMs of this type [73,74] have been used to generate primary tumor tissues to derive low passage, genetically defined tumor cell lines, providing a facile platform for in vitro phenotypic drug discovery screening of NCE. In addition, these low passage oncogenically modified tumor cultures form the basis for orthotopic engraftment into the native colonic microenvironment of immune-competent mice to effectively and reproducibly model tumor-stromal interactions and enable the stringent evaluation of novel therapies for effects on primary and metastatic tumor development in an in vivo setting that recapitulates the progression of human colorectal cancer [73,74]. Furthermore, these types of GEMMs have been reported to be predictive of eventual therapeutic response in human clinical trials [72–75]. These models lend support to the axiom that, despite caveats in the results achieved from any pre-clinical model, tumor models that carry the genetic and phenotypic signatures of the native cancer can recapitulate clinical phenotypes to a major extent, including therapeutic responsiveness and the development of therapeutic resistance.

Further examples of the utility and translational impact of specific GEMMs extend to other types of cancer as well. Studies using oncogenic *Cre-Lox/K-Ras* driven GEMMs of NSCL and pancreatic cancers have demonstrated that these models can recapitulate therapeutic responses, or the lack thereof due to tumor stromal components, to a variety of standard-of care targeted therapeutic agents, including inhibitors of EGFR and VEGFR, as well as traditional cytotoxic agents [32,67,70,71]. While in several instances discussed above in regard to ectopic tumor cell line derived xenografts, inhibitors of oncoprotein "drivers" such as EGFR, ALK, and BRaf were predictive of human therapeutic response to these therapeutic agents, GEMMs offer the advantage of studying these agents within the context of critical tumor-stromal interactions and a functional humor and cellular immune system in the host animal, conditions more directly relevant to understanding the effects of therapies on tumor invasion and metastasis, angiogenesis, and the emergence of tumor resistance. These processes are associated with unique tumor-micro environmental interactions not achievable in an ectopic tumor xenograft model in an immune-compromised host. Similarly, it is imperative to reiterate the translational applications of conditional transgenic GEMMs both in the identification and characterization of tumor-specific biomarkers in plasma and tumors within a more relevant tumor-stromal microenvironment, and for assessing therapy-associated changes in specific signaling pathways that are directly relevant to the phenotype of the corresponding human malignancy [13,32,70,73,74].

Despite the advantages and utility of using GEMMs, these models require a great deal of time, effort and cost to develop, establish, and maintain the necessary numbers of genetically engineered animals to produce statistically meaningful data, often due to heterogeneity of tumor incidence and progression. Moreover, anti-tumor efficacy studies in these models can last weeks or months in duration to achieve the desired primary and secondary endpoints. Unlike traditional ectopic xenografts in which tumors can be readily measured quantitatively using

electronic calipers, quantitative assessment of the impact of therapeutic agents on tumor growth and metastatic progression in GEMMs (as noted above for orthotopic xenograft models) in situ relies on the use of imaging modalities, including magnetic resonance imaging (MRI), computed and positron emission tomography (PET), Doppler ultrasound, and bioluminescent imaging – all of which have distinct advantages and limitations [13,33,67,68]. While providing essential data and saving on the number of animals needed per cohort of a therapeutic efficacy study, these imaging modalities must be considered with regards to their resolution, whether they provide any functional information on tumor biology, their degree of invasiveness to the host animal, and the duration of each imaging session – all of which are technical and practical considerations in the context of an in vivo screening paradigm. In addition, these modalities come at a high initial capital cost –$250,000 to over $1,000,000 [33,67,68] which can be prohibitive for many laboratories. Consequently, the use of transgenic GEMMs is optimal in the mid-to-latter stages of a drug discovery project where optimized leads or pre-clinical development NCEs can be evaluated in specific, clinically relevant GEMMs to more effectively support the translational process in terms of determining tumor-specific biomarkers, understanding the molecular determinant(s) of the clinical response, and better understanding of potential therapeutic resistance mechanisms associated with novel therapeutics.

2.4. Primary tumorgraft models of human solid tumors in immunocompromised mice

Primary tumorgraft models (also referred to as personalized tumorgrafts and avatars) are among the most recent class of preclinical cancer models and involve the direct implantation, serial transplantation and propagation of freshly excised (within several hours of surgery) primary human tumors into immune-deficient mice to create a primary human tumorgraft in a manner that preserves and stabilizes both the genotypic and phenotypic features of the original human tumor. These models were first reported a decade ago [75] and afford a powerful, experimentally rigorous and more clinically predictive approach to oncology drug discovery and development that has become increasingly refined in recent years [76–79]. Utilizing this approach avoids potential secondary molecular and epigenetic changes and artifactual growth profiles of tumors that can occur after long periods (sometimes decades) of in vitro selection and expansive growth in enriched cell culture systems under non-physiological environmental parameters as discussed above in relation to the limitations and disadvantages of ectopic xenografts and orthotopic xenograft models derived from established tumor cell lines. Moreover, an additional advantage of these primary tumorgraft models is the absence of in vitro selection of tumor cells, maintenance of the primary tumor cell architecture and the retention of the inherent genetic heterogeneity of the original tumor, with primary tumorgrafts capturing most of the genome-wide variants observed in the original human tumor [75–79]. In addition, low in vivo passaged tumorgrafts in many instances retain the tumor stromal cells and cancer stem cell-like components that comprise the original primary tumor and can be characterized for their molecular, biochemical and histological profiles of interest. Grafts can be either serially passaged exclusively in vivo or cryopreserved and banked after a limited number (three or four) of serial in vivo passages [77,78]. Importantly, orthotopic implantation of primary tumorgrafts offers the additional advantage and physiological relevance of maintaining their clinical markers and histopathologies, gene expression and DNA copy number variations, while frequently recapitulating clinically relevant patterns of metastasis [78,79]. In this regard, primary sc implanted tumorgrafts with the capacity for spontaneous metastatic dissemination to clinically relevant organ sites have also been described [80]. These sc implanted primary tumorgrafts with the capacity to metastasize are a marked contrast to the poor or absent metastatic ability of ectopic sc or ip implanted tumor xenografts generated from in vitro selected and heavily passaged tumor-derived cell lines as discussed above. Low serial passage primary tumorgrafts are stable, and do not change appreciably with respect to their growth profiles, histology, and gene and protein expression profiles [76,78], again a considerable advantage for consistency and reproducibility in the in vivo pharmacological assessment of novel or established therapeutics when compared to studies in ectopic tumor cell line-derived xenografts or tumor cell line-derived orthotopic models.

More importantly for their utility as preclinical drug discovery models is the clinical relevance and predictability of primary tumorgrafts to a variety of cytotoxic and targeted therapeutic agents. Primary patient-derived tumorgrafts benchmarked against a variety of standard of care chemotherapeutic agents demonstrated that these primary tumorgrafts correctly predicted positive clinical responses in 90% (19/21 tumors) and therapeutic resistance in 97% of patients (57/59 tumors; [75]). More recent studies [77–79] confirmed and expanded these observations supporting the application of primary tumorgrafts as being reflective and predictive of clinical responsiveness or resistance of patient tumors to specific therapeutics that would not have necessarily been selected as a standard treatment of choice for a particular cancer. A pilot study in 14 patients with refractory advanced cancer whose treatments were selected on the basis of activity against primary tumorgrafts developed from the patient's own cancer illustrates this concept. Tumors resected from these patients were propagated in immune-deficient mice and treated with 63 drugs in 232 treatment regimens. The treatments selected for each individual patient tumorgraft were not necessarily the first choice for a conventional second- or third-line cancer treatment. The objective response rate was 88% for treatments deemed effective in these tumorgraft models and tested in the patients, with 11/14 patients achieving a partial objective response [79]. The strength of these preclinical-clinical correlations of tumor responsiveness further support the utility of tumorgraft models as being highly predictive of clinical outcome [78,79]. In this regard, the abundant tumor materials obtained by serially propagating tumorgrafts in mice allow for in-depth biochemical, molecular, and pharmacological profiling to identify potential biomarkers or gene signatures in response to specific therapies, allowing the development of rational drug combination approaches, or guiding new therapeutic approaches in specific cancers. An illustration of the potential of this personalized medicine approach utilizing a patient's primary tumorgraft to guide therapy was the outcome of a patient with advanced, gemcitabine-resistant pancreatic cancer who was treated with the DNA damaging agent mitomycin C based on the observation of significant activity of this class of drugs against a personalized tumorgraft generated from the patient's surgically resected tumor [81]. Contrary to the expected median survival of 3 months for pancreatic cancer patients who progress on gemcitabine, this patient remained symptom free for over 3 years after receiving mitomycin C, a treatment regimen that would not have been used conventionally as a second-line therapy for gemcitabine-resistant pancreatic cancer. Deep sequencing of virtually the entire coding region of the genome in this patient's tumor revealed biallelic inactivation of PALB2, a gene involved in homologous recombination repair of DNA damage, the loss of which conferred synthetic lethality of the patient's tumor to this treatment regimen [81]. Studies of this nature highlight the translational applications of global tumor genomic sequencing approaches when combined with the use of primary tumorgraft models. As the ability to obtain global genomic information from

individual patient tumors becomes increasingly greater and more facile, primary tumorgrafts with corresponding clinical responses from the patient from which they are derived will become a viable platform to systematically explore the therapeutic relationships between drug response and tumor specific genetic and epigenetic alterations conveying sensitivity or resistance to specific treatments [78,79,81] and may facilitate the identification of novel synthetic lethality therapeutic approaches in specific cancers as well.

Despite their inherent power, physiological and clinical relevance, and predictability of response, primary tumorgrafts also have inherent limitations and disadvantages. Generating primary tumorgrafts requires access to freshly excised, quality primary tumor materials from patients, together with considerable laboratory resources to process and generate the tumorgraft through serial passaging and profiling in the murine host. Even in the best conditions, 25–30% of primary tumor implants fail, and those that engraft after several months time often require 6–8 months of serial in vivo propagation to be useful for banking as cryopreserved primary tumorgrafts [78,79]. Viable low passage cryopreserved tumorgrafts must be validated for their ability to be re-established in mice, and their growth properties, histological, molecular and genetic profiles confirmed to ensure the consistency and reproducibility of the tumorgraft both to the initial mouse graft and the original primary tumor prior to its routine use in drug discovery research. Consequently this process demands considerable front-end costs and labor-intensive preparations, impacting the efficiency, speed, and cost of using primary tumorgrafts in early stage drug discovery screening paradigms. However, once such models are established and subsequently validated, or if a commercial provider of tumorgraft technology is utilized [see 80,82,83], their use can be invaluable in the mid-latter stages of a drug discovery project to facilitate the rigorous evaluation of advanced optimized leads, or to characterize the profile of pre-clinical development candidate NCE advancing into clinical trials. Given the predictive potential for clinical responsiveness, primary tumorgrafts have effectively become avatar models of human cancer in which to conduct synchronous 'co-clinical trials' of novel therapeutic agents similar to studies conducted presently with specific GEMMs [27,28]. However, while primary tumorgraft models may successfully enable preclinical research, drug discovery and genetic and proteomic biomarker evaluation, their utility in a direct personalized medicine approach to cancer therapy may be severely limited by the months required to establish the model such that the patient may die long before his/her tissues are ready for use in a pre-clinical experimental setting.

2.5. Carcinogen-induced multi-stage models of tumorigenesis in immuno-competent rodents

Single and multi-stage carcinogen-induced models of various solid and hematological cancers are among the oldest and most diverse pre-clinical models used in cancer biology. These models effectively recapitulate the time-dependent and multi-stage progression of tumor pathogenesis in response to etiologically relevant environmental carcinogens and tumor-promoting agents [19]. These models utilize the topical, ip, or po administration of a variety of polycyclic aromatic hydrocarbon carcinogenic agents (e.g., N-nitroso-N-methylurea (NMU), N-methyl-N-nitro-N-nitrosoguanidine (MNNG), 7,12-dimethyl-benz(*a*)anthracene (DMBA), azoxymethane (AOM)), and tobacco-smoke associated carcinogens and nitrosamines including benzo(*a*)pyrene (BaP), diethyl-nitrosamine (DEN), 4-methylnitrosamino-3-pyridyl-1-butanone (NNK), and N-nitrosobis(2-oxopropyl) amine (BOP) in established protocols either alone or in combination with known tumor promoter agents, e.g., phorbol esters, to induce specific cancers in a variety of immune-competent mice and rodent strains. The susceptibility to chemical carcinogen-induced cancers and the resultant tumor incidence and multiplicity varies with the protocol, dosage and schedule of carcinogens and promoters, and the age and particular strain of rodents used. The more widely utilized and best understood carcinogen-induced pre-clinical models in both immune-competent mice and rat strains include: the NMU (N-nitroso-N-methylurea)-induced mammary carcinoma model [84,85], the DEN (diethylnitrosamine)-induced hepatocellular (HCC) carcinoma model [86], NMU- and MNNG (N-methyl-N-nitro-N-nitrosoguanidine)-induced gastric carcinoma models [87], BOP (N-nitrosobis(2-oxopropyl)amine)-induced pancreatic ductal carcinoma models in hamsters [88], AOM (azoxymethane)-induced colorectal carcinoma models [89,90], NNK (nitrosamine 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone)-induced lung carcinoma models [91,92], and DMBA-(7,12-dimethylbenz[*a*]anthracene) and BaP (benzo[*a*]pyrene)-induced squamous cell carcinoma (SCC) models of the skin and upper aerodigestive tract in rats, mice and the hamster oral mucosa [93–95]. With respect to the latter class of models, because of easy accessibility and similarities with head and neck epithelia, mouse skin/epidermal carcinogenesis models using DMBA, BaP, NNK and various nitrosamines have frequently been used in translational studies to evaluate the pathobiology and treatment of tobacco-associated SCC [19,94,95].

As a general class of pre-clinical cancer models carcinogen-induced tumor models offer a variety of distinct advantages and clinical relevance to human cancers. Generally, these models are generated in out bred rodent strains of various genetic backgrounds, produce a high incidence of organ-specific lesions, and are highly reproducible in their phenotype. Additionally, their biological utility and clinical relevance lies in their molecular, biochemical, and histopathological similarities to the developmental sequelae of specific human cancers, from hyperplasias, dysplasias, pre-malignant lesions (for example, papillomas and adenomas), low grade well-differentiated carcinomas, and ultimately to invasive and more poorly differentiated carcinomas capable of metastasizing to varying degrees both locally and to distal organ sites in the host [19,87–89,92,94,95]. Consequently, these types of models are invaluable in investigating which stages of tumor development are most sensitive to therapeutic and or preventive intervention with novel or known therapeutic agents. Depending upon the specific models, carcinogen-induced tumors can be readily measured quantitatively using electronic calipers as with traditional ectopic sc xenografts, or in the case with GEMM and orthotopic xenograft models, they may rely on various imaging modalities to quantitatively assess in situ of the impact of therapeutics on tumor growth and metastatic progression. As with GEMMs and both traditional ectopic xenografts and orthotopic tumor models generated in syngeneic hosts, carcinogen-induced tumor models enable the assesment of the role, and modulation thereof, of cellular and humoral immune-components in tumor immune evasion and surveillance mechanisms, and the contributing role of acute and chronic inflammatory processes in tumor development and progression [86,87,89–92]. The latter is particularly relevant given the experimental and epidemiological association between chronic inflammation and tumor development in humans [1,96]. In terms of their clinical predictability of responsiveness in humans, the practical use of these models to study therapeutic effects on tumor growth and development have been dominated by cancer chemoprevention studies with fewer utilizing these animal models to evaluate therapeutic effects. Several experimental agents found to be beneficial in the prevention or management of specific human cancers are highly efficacious in their corresponding organ-specific carcinogen-induced cancer models [89,91–94].

Despite the clear utility and advantages of carcinogen-induced models, these have inherent limitations and disadvantages for routine use in the early stages of drug discovery. Although requiring minimal manipulation and marginal initial costs to implement, the prolonged time frames and associated costs in animal maintenance and care inherent in conducting these models on a routine basis is a factor for consideration. For example, depending on the carcinogen, dose schedule, and rodent strain, inducing tumors in these models at frequencies of 80% or greater can require a minimum of 5–6 weeks for induction of mammary carcinomas (e.g., NMU-induced mammary cancer in rats [84] to 40–50 weeks in AOM-induced colon carcinomas in rats [89]), although abbreviated protocols of AOM-dextran sulfate, colitis-induced colon tumors have been described in mice that require only 5–6 weeks of induction [90]. Similarly, 24–40 weeks are required depending upon the specific protocol for induction of BOP- and other nitrosamine-induced pancreatic carcinomas in hamsters [88], NNK-induced lung tumors in mice [91,92] and DEN-induced HCC tumors in mice [86]. In the widely used DMBA-phorbol ester induced two-stage model or BaP-induced complete carcinogenesis models of head and neck SCC (squamous cell carcinoma) and upper aerodigestive tract cancers, all types of SCCs from well- to poorly differentiated can be reproduced, similar to the human situation, but comparably long time frames relative to murine life spans are required for the development of these lesions. For example, manifestation of papillomas in 100% of animals can be achieved within approximately 15 weeks, and development of SCC occurs in upwards of 90% of the animals after 30 weeks of DMBA-phorbol ester induction in moderately sensitive mouse strains. Induction of BaP-induced invasive SCC with a comparably high frequency requires the repetitive topical application of BaP for up to 50 weeks [91–95]. In general, although there are exceptions, these timelines and the associated safety and handling concerns for the preparation and long term use of various carcinogenic agents or combinations of carcinogens and promoters in animal studies, are practical limitations to the routine, facile use of these models in early-mid stage drug discovery efforts. Nonetheless their histological, molecular, and phenotypic similarities, their general propensity to metastasize, and their use in studying immune and inflammatory components in the multi-step sequelae of tumor development and progression, make these tumor models valuable translational biology systems for the evaluation of NCEs in the post-drug discovery – early non-clinical development stage or when NCEs have entered clinical trials.

3. Translational challenges, prospects and conclusions

It is critical to recognize that each type of animal model of cancer has intrinsic advantages and limitations leading to practical considerations for their effective use in drug discovery in the elucidation of the mechanisms and regulation of tumor initiation and progression and in the responsiveness of a particular tumor type to specific therapeutic agents. Accordingly, each of the particular classes of pre-clinical tumor models, and the data generated with them, should not be viewed in isolation or as an absolute predictor of the human response to a novel therapeutic agent. Rather, the specific classes of models detailed in this review for their strengths, weaknesses, utility, and clinical predictive value in oncology drug discovery, should be viewed as a portfolio of sophisticated biological tools that can be utilized optimally at various stages in the drug discovery process to answer specific experimental questions. Likewise, the data sets generated from each type of model also need to be evaluated collectively and in the context of the complete profile of an NCE emerging from drug discovery programs. Evaluating NCEs in a variety of different types of tumor model systems with different genetic and phenotypic attributes can also provide a better representation of the genetic and epigenetic heterogeneity and resiliency that are a hallmark of human cancers.

The decision of what types of models to use, how many, and at what stages in the iterative drug discovery process is directly dependent on the specific questions being addressed. It is important to recognize that classes of models can be used to varying degrees at multiple stages in the drug discovery process and beyond, dependent upon the molecular target or pathways being investigated (Fig. 1; Table 1).

3.1. What is needed from a preclinical tumor model(s) to be used early in the drug discovery process?

Defining tumor PD/PK relationships for NCE against a particular molecular target or signaling pathway and determining the direct effects of an NCE on anti-tumor efficacy and tolerability upon repeat administration to a tumor bearing animal, are questions that can be readily addressed in a facile and rigorous manner with one or more ectopic tumor xenograft models either in immune-compromised or syngeneic hosts. These same types of models can also be used subsequently to explore dose scheduling paradigms and their impact on the magnitude and duration of tumor PK/PD effects and anti-tumor efficacy. In terms of the NCE scheduling paradigms are key questions that include: is the NCE only effective in attenuating tumor growth when it is present?; Can it reverse tumor growth or only prevent additional growth?; Are its effects dose/plasma concentration and frequency of dosing dependent related?; Is it as effective in terms of its PD effects and dose-related potency as a current standard of care drug?; Does cessation of treatment with the NCE cause an immediate rebound in tumor growth or is there a lag phase before regrowth?; Do the effects of the NCE tolerate – either during treatment or following a "drug holiday"?; Does the NCE cause profound, dose-related weight loss or other toxicities?

Of course, if the molecular target or pathways under investigation are implicated in tumor-stromal interactions and directly related to the emergence of tumor invasion, metastasis, angiogenesis, or immune surveillance and evasion mechanisms, then the use of orthotopic models, particularly in a syngeneic hosts are essential to incorporate early in a drug discovery flow once tumor PK/PD or an initial assessment of in vivo efficacy and tolerability have been established, for example with panels of ectopic tumor xenografts. For NCE advancing to the next level of a drug discovery flow based upon not only their initial tumor PK/PD and anti-tumor efficacy profiles, but additional criteria achieved with respect to their PK and metabolic profiles, pharmaceutical properties, target selectivity and off-target activities, etc., a next tier of more rigorous, predictive, and clinically relevant tumor models should be employed. These would include specific GEMMs depending upon the molecular target or mechanisms under investigation, and primary human tumorgrafts (both sc and orthotopic) bearing the genotypic and phenotypic profile relevant for the molecular target or mechanism under investigation. Profiling more advanced NCEs emerging from the mid-to-later stages of a drug discovery flow in these types of models affords not only greater rigor and clinical relevance, but a better understanding of the effects of the NCE on tumor-stromal interactions impacting immune surveillance mechanisms, localized tumor invasion and systemic metastatic spread, tumor angiogenesis, and the mergence of acquired therapeutic resistance. In the later stages of drug discovery projects where pre-clinical development candidate NCEs are being defined based on a multitude of criteria, and in the post-discovery stage where pre-clinical candidate NCEs have been identified and are advancing into non-clinical development activities, the expanded use of additional and more

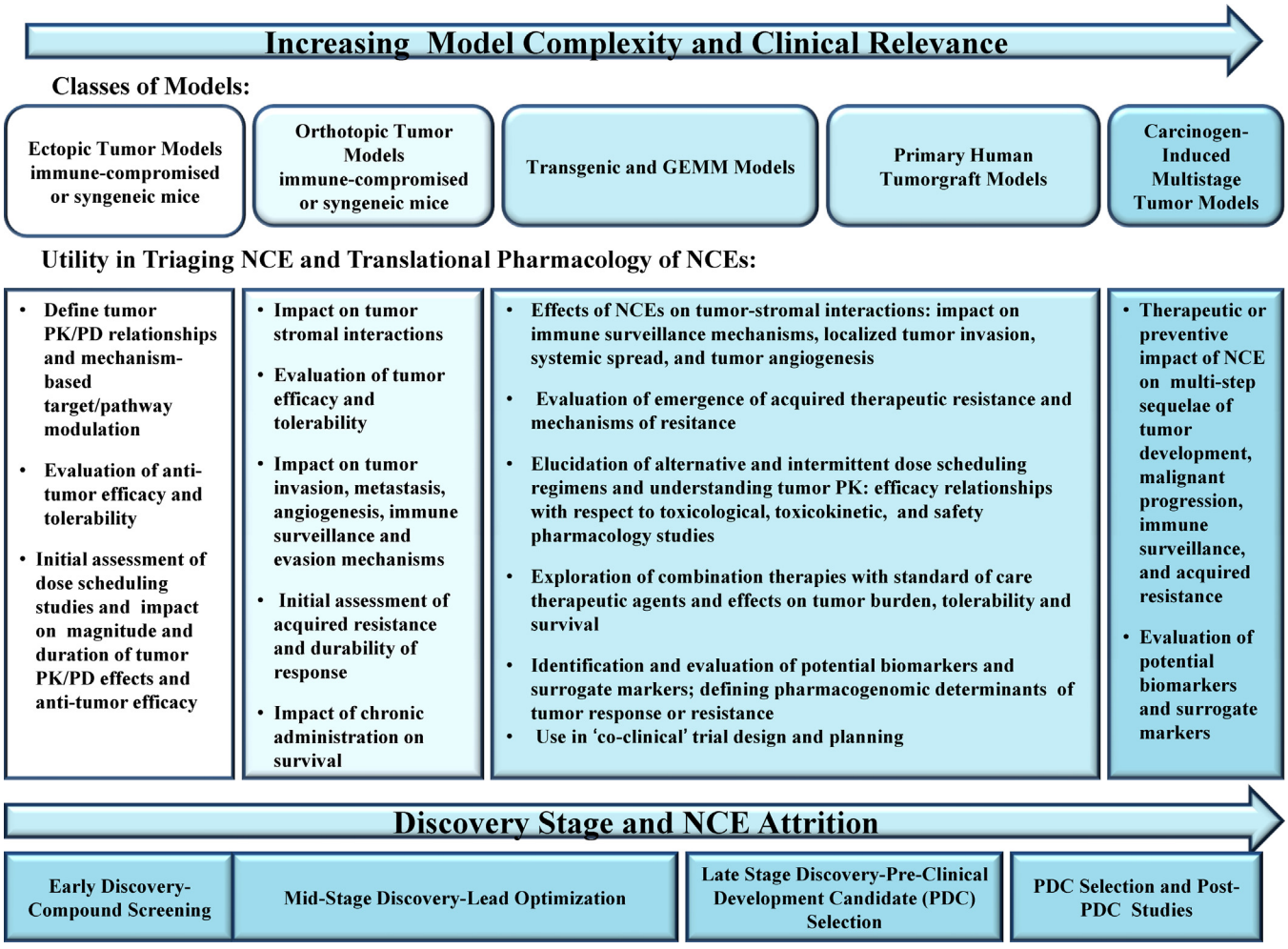

Fig. 1. Applications and utility of specific types of pre-clinical tumor models for drug discovery and translational biology.

specialized GEMM and primary tumorgraft models are invaluable when used pragmatically in a hierarchy of increasing complexity and physiological relevance. At this juncture, evaluating an NCE using several of these types of models can provide a substantial translational pharmacology dataset to help guide clinical development, including: (i) helping to elucidate alternative dose scheduling regimens and understand plasma and tumor PK: efficacy relationships in relation to ongoing or planned toxicological and safety pharmacology studies in rodents and non-rodent species; (ii) exploring combinatorial therapies with established standard of care therapeutic agents for their efficacy (and tolerability) profiles against primary and metastatic tumor growth and their effects on survival (Kaplan–Meier analyses [66]); and (iii) identifying and evaluating potential tumor-specific biomarkers and surrogate markers to understand the molecular and pharmacogenomic determinant(s) of potential clinical responses to the NCE. Finally, depending upon the nature of the molecular target or mechanisms and the type of cancers being targeted with the NCE, the use of a suitable complete or multi-stage carcinogenesis protocol in the post-discovery/non-clinical development phase of the NCE in a tumor model that recapitulates multiple phenotypic aspects of the corresponding human cancers can provide valuable translational insights as to which stage and to what degree and duration the NCE therapeutically impacts the multi-step sequelae of tumor development and malignant progression in a fully immune-competent host.

Despite the sophistication and physiological relevance of even the most predictive and clinically relevant human cancer models (for example, GEMMs and primary human tumografts), the ultimate proof of concept for efficacy and safety of novel oncology therapeutic agents lies in humans. Hence, a greater emphasis needs to be given to translational medical research in early stage clinical trials where the clinical experiment with an NCE undergoes real time iteration with pre-clinical research to improve ultimate clinical outcomes. An important consideration in this regard is the fact that dosing regimens, formulations, and schedules of therapeutic agents employed in the clinic rarely correspond to those used in pre-clinical stages of oncology drug discovery [26]. This fact has prompted the use of multi-institutional synchronous 'co-clinical trials' in mice as noted above, especially in GEMMs (and currently in primary tumorgraft models) in glioblastoma [27] and NSCL cancers [28] to evaluate and optimize the use of targeted cancer therapies. In this scenario, the tumor-bearing animal is treated with a specific agent and dosing regimen and evaluated in a parallel manner to the corresponding human clinical protocol, necessitating real-time integration of human and murine data with regards to efficacy, tolerability, PK/PD relationships, and genetic and biomarker assessments. Although this newer approach to personalized medicine is still in its relatively early stages, it has impacted screening protocols for patient selection, assessment of specific biomarkers and surrogates, and helped define specific primary and secondary end points in clinical protocols. The ultimate objective of this 'co-clinical trials' approach is to tailor future treatment regimens for the cancer patient and bridge the translational gap between the clinic, pre-clinical tumor models, and the discovery and development of new therapeutic agents.

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