Clinical Trial Perspective

CLINICAL INVESTIGATION

Can mouse models of cancer reliably improve clinical trial outcome?

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One of the major threats humankind faces today is cancer. Incidences of neoplasia worldwide are estimated to translate to 13 million cancer deaths by 2030. Despite promising response rates through treatment modalities such as surgical resection as well as chemo- and radiation therapy in a few cancer entities, we still lack sufficient therapeutics that provide long-term survival in cancer patients, especially in patients with advanced disease. Hence, accelerated translation of highly encouraging *in vitro* and *in vivo* preclinical therapeutic findings is urgently needed to meet the demand for novel cancer drugs to combat cancer mortality successfully. Genetically engineered mouse models recapitulating characteristics of human disease have become an indispensable tool in cancer research to predict clinical outcome. This commentary highlights current benefits and limitations of developing novel mouse models of cancer to subsequently improve clinical trial outcome in humans.

Keywords: cancer • clinical outcome • co-clinical trial • drug intervention evaluation • genetically engineered mouse models • mouse avatars • patient-derived xenograft models • preclinical animal models • xenograft models

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In 1953, Watson and Crick paved the way for present day cancer research by unraveling 'the secret of life' – the DNA double helix. All functional processes of life are encoded by DNA and the complete set of this information is referred to as an organism's genome. During the decades following this biological revolution, enormous research efforts revealed that cancer is a collection of different, constantly evolving diseases caused by alterations within the human genome [1]. The development from an incipient tumor cell to the final cancerous state involves the acquisition of multiple sequential mutations – these are either genetic and/or epigenetic lesions [1]. As a result of these vested mutations, cancer cells obtain a set of traits that have been termed the 'hallmarks of cancer' – potential for unlimited proliferation, mitogen-independence, escape from apoptotic signals, immune evasion, sustained angiogenesis, tissue invasion, metastasis, reprogramming of energy metabolism and evading immune destruction [1,2]. Although advanced genome technologies are widely employed to characterize the underlying molecular mechanisms of cancer in greater detail, cancer researchers remain challenged to develop highly relevant and predictive models of human disease to ultimately improve patient care and treatment. In particular, the necessity for improved animal models and better designed preclinical trials is further exacerbated by the low probability of translating research evidence from animals to humans [3]. In their systematic review, Hackam *et al.* found that only a third of highly cited animal research studies were translated at the level of human randomized trials and approximately a tenth of the interventions were approved for patient use [3]. Kola and Landis report similar results and highlight distinct differences in success rates between various diseases [4]. Approximately

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5% of novel antineoplastic compounds were eventually licensed following demonstration of robust clinical performance in Phase III trials, whereas the success rate for cardiovascular diseases was as high as 20% [4]. It remains tempting to speculate whether the limited effectiveness of successful translation is less the result of the incomplete nature of the applied animal models than of inadequate preclinical studies and misinterpretation of the models. Here the authors focus their discussion on cancer models in the laboratory mouse and highlight efforts to improve existing rodent and human genetic models to result in more predictable clinical outcome, prior to conducting enormous, expensive clinical trials.

Role of murine models in cancer research

Traditionally, the laboratory mouse (*Mus musculus*) has served as a versatile tool in mammalian genetic research. Attractive features of this model system include: mice are small, easy to maintain and straight forward to breed in captivity, and most importantly, they share approximately 99% of their genes with humans, and their genome is amenable to manipulation. Despite these invaluable advantages, every model system is simplified and reduced in its variables by nature and therefore needs to be considered within the range of its validity. In keeping with this definition, a thorough experimental validation of a distinguished genetic alteration must be the sole variable in an otherwise stable environment. In stark contrast, only a minor amount of human conditions are based on a single genetic alteration. For instance, the analysis of adult breast, ovary, colorectal, pancreas and glioma cancer genomes showed an average of 1000–10,000 somatic mutations [5,6]. Consequently, cancer researchers are forced to employ 'imperfect tools' to study complex biological processes. However, the diversity of rodent models available for cancer research illustrates that not every limitation holds true for any animal model, and a close evaluation of the favored experimental set up is required to obtain valid results. One of the major goals in animal-based cancer research is the evaluation of potential novel drugs to improve patient outcome. Whether the laboratory mouse represents an ideal platform for this purpose still remains controversial. Although *M. musculus* and *Homo sapiens* share 99% of common genes, significant species differences including physiology, anatomy, metabolism, biochemistry, pharmacokinetics (PK) and toxicokinetics are important factors to be considered in the attempt to compare the mammalian systems. The relevance of species-specific characteristics in PK and toxicokinetics becomes even more evident as numerous clinical studies describe the prevalence of intraspecies alterations in humans, influencing the metabolism of therapeutic agents. Gender, age, health status and ethnicity of the examined patients were predominantly associated with variable drug response [7–9]. Pursuing this further, the experimental data set of Fraga *et al.* shows that even monozygotic twins display distinct differences in drug metabolism [10]. Hence, generalized international standards taking into account the mediators of intraspecies variability are needed to achieve higher predictability of clinical outcome in mouse models of human disease. In keeping, several studies demonstrated that the effectiveness of drug regimens established in mouse models correlates only infrequently when performed in the clinic [11–13]. Although the aforementioned aspects might lead to the assumption that mouse models are not appropriate predictive systems, the current limitations might be merely technical and could be overcome to accurately predict clinical outcome in drug intervention trials. Several recent studies demonstrate that specific mouse models are capable of accurately recapitulating the response and resistance observed in the clinic [14–16]. Below, the authors will discuss the latest advances in generating rodent models for most reliable preclinical treatment evaluation (summarized in **Table 1**).

Benefits & limitations of cancer mouse models in therapeutic assessment

Today's antineoplastic drug development and efficacy studies highly rely on murine model systems. The most established model in cancer research includes subcutaneous implantation of cultured human (xenografts) or mouse (allograft) cells, or tumor tissue explants into immunocompromized or immune-competent host mice, to mimic tumorigenicity and treatment response in a complex biological system. This method is widely used because of its easy application, rapid and large tumor cohort generation, and simple preclinical data assessment. Nevertheless, considerable concerns need to be addressed regarding the accuracy of xenograft implants to serve as a surrogate for tumorigenesis and drug response. The latter especially remains a topic of dispute as the preclinical efficacy of anticancer activity shown for various novel agents in xenograft models failed to translate into improved clinical outcome [17,18]. Potential explanations for this disappointment are as follows: first, most of the applied tumor cells have been cultured *in vitro* prior to injection and consequently tend to grow rapidly. Conversely, the doubling time of most human cancers is much slower [19]. Thus, the sensitivity to chemotherapeutic agents targeting dividing cells might be exaggerated. Second, for the sake of ease, the vast amount of experimental cells is injected ectopically into the flanks of the murine host. However, as early as 1992, the studies conducted by Wilmanns *et al.* demonstrated that the therapeutic response of cancerous cells highly depends on the surrounding tumor environment [20].

Table 1. Comparative overview highlighting benefits and limitations of available murine models for cancer research.

Model	Availability			Accuracy of tumor biology					
	Generation of model	Costs	Induction of tumor growth	Origin of tumor	Tumor evolution	Genomic profile and stability	Tumor micro-environment	Immune system	Metastatic disease
Conventional xenograft models	Quick; days to weeks	Low	Ectopic tumor implantation: simple technique, subcutaneous injection Orthotopic tumor implantation: depending on target organ more advanced skills required	Murine/human cancer cell lines, no tumor architecture	Unnatural course, transplant	*In vitro* culturing induces multiple additional mutations accelerating unleashed proliferation, genetic profile rarely correlates with human counterpart	Surrounding tumor stroma of different species; ectopic vs orthotopic implantation	Most often none (host is immuno-compromized); advancement: co-transplantation of human hematopoietic stem cells to establish a functional immune response	None
PDX	Depending on tumor entity 6–12 months	Fair	Fresh tumor samples required Ectopic tumor implantation: advanced skills for subcutaneous transplantation required Orthotopic tumor implantation: depending on target organ major surgical skills required	Patient-derived tumor tissue, partially retained tumor architecture with supporting stromal cells	Unnatural course, transplant	Genetic profile as parent human tumor, genomically stable for four tumor generations	Surrounding tumor stroma of different species; ectopic vs orthotopic implantation	Most often none (host is immuno-compromized); advancement: co-transplantation of human hematopoietic stem cells to establish a functional immune response	None
GEMMs	Labor intensive; up to several years	High	Depending on conditional manipulation system (includes simple and further advanced techniques)	Autochthonous	Autochthonous initiation to progression of tumor	Recapitulates cancer-driving mutations of human disease, genomically stable	Surrounding tumor stroma and vasculature provided by host organism	Functional immune system provided by host organism	Only observed in very few models

GEMMs: Genetically engineered mouse models; PDX: Patient-derived xenografts.

Third, coupled with this notion, rising evidence stresses the significance of immune cell interaction and the tumor microenvironment on tumor initiation, maintenance and response to treatment [21]. To prevent hyperacute rejection of xenograft implants, the experimental setup requires use of immunodeficient recipients. However, there is an alternative view: this supposedly 'imperfect' animal model proved to be the ideal system to evaluate the direct effects of humanized monoclonal antibodies. Three prominent examples of successfully translated immunotherapeutic anticancer treatments are trastuzumab, bevacizumab and cetuximab.

At this point, the utilization of human xenografts resembles a Janus-faced undertaking in predicting clinical outcome of antineoplastics and careful experimental planning is necessary to fulfill valid hypothesis-driven cancer research. Nevertheless, over the past 10 years the dreadful reputation of xenograft models of cancer has changed drastically by virtue of implantable primary human tumorgraft models. In comparison to standard xenografts, which employ permanent cell lines, this animal system is based on direct transfer of explanted tumorgrafts. The subcutaneously implanted tumor tissue fragment is smaller than a pencil eraser and contains – apart from malignant cells – supporting stromal tissue, and therefore mirrors the anatomically correct tumor architecture. The potential of these patient-derived xenograft (PDX) models in drug candidate screening was further underscored by the analysis of Fiebig *et al.* [22]. In addition to correctly predicting therapeutic response in 90% of patients, the employed PDX even validated a prognosticated resistance of 97% [22]. The recent pilot study published by Hildago *et al.* clearly confirmed the power of PDX to anticipate personalized anticancer treatment [23]. In total, samples of 14 patients with refractory advanced cancers were engrafted and treated (63 drugs in 232 treatment regimens), which resulted in identification of effective treatment regimens for 12 patients [23]. This successful translation into the clinical setting is most likely attributed to the missing intermediate step of *in vitro* culturing, which usually induces genetic changes in tumor cells to become less differentiated and more homogeneous. PDX, however, reflect the parent human tumor more faithfully.

Nevertheless, PDX are also not immune to genetic drift if serially passaged in animal hosts. The recent genomic analysis of matched patient tumors and explant tumorgrafts performed by Monsma *et al.* revealed that the transcriptomes and oncogenic mutations found in human tumors were stable for four tumorgraft generations [24]. These results and similar findings of others suggest that the genomes of PDX are stable and thus robust enough to evaluate novel treatment strategies [25–27]. Eventually, these promising data derived from PDX culminated in terming these personalized tumorgraft models 'mouse avatars' [101].

Despite the pioneer spirit of 'avatar mice' to potentially account for a paradigm shift in cancer patient treatment, there are still several serious drawbacks. To begin with, neither the injection of permanent cell lines nor the implantation of patient-derived tumorgrafts is capable of fully recapitulating the initiation-to-progression course of the disease. Along these lines, the majority of PDX are still engrafted ectopically (with the exception of melanomas). The significance of this 'non-orthotopic' PDX propagation is not completely understood yet. In comparison with the previously outlined studies by Wilmanns *et al.*, which demonstrated strict dependency of adequate orthotopic tumor environment and subsequent therapeutic response in a xenograft context [20], the high correlation of therapeutic outcome despite ectopic PDX implantation in Fiebig's experiments contradicts these findings [22]. Further evidence refuting the relevance of orthotopic PDX growth for drug discovery and development was recently provided by Monsma *et al.* who demonstrated stable expression profiles of the parent 'orthotopic' malignancy in subcutaneous primary PDX [24]. However, the inappropriate anatomical site for tumorgraft implantation might potentially account for the commonly observed transplantation failure rate, which can be as high as 97%, depending on the cancer entity. The most obvious explanation of this phenomenon seems to lie in the lack of autochthonous stromal tissue to support tumor growth. DeRose *et al.* approached this problem by co-injection of primary human mesenchymal stem cells (MSC) together with tumorgrafts of breast cancer patients [28]. They report that the addition of MSC results in phenotypic stability of the grafts and also enhances growth of existing tumors by promoting angiogenesis [28]. Although it is plausible that MSCs would enrich the microenvironment of the mouse mammary gland with human growth factors, proangiogenic factors and various tumor-promoting chemokines [29], DeRose and colleagues failed to increase the 'transplantation take rate' by concomitant implementation of MSCs in tumors that had also not grown without MSCs [28]. In line with the 'seed and soil' theory of cancer metastasis, it remains to be seen whether prior establishment of a 'tumor-enhancing microenvironment' helps to augment the tumorgraft 'take rate.' Furthermore, the key players in tumorgraft growth support have not been identified yet.

Lastly, propagation of a single human tumor into multiple mice forming a valid testing platform takes up to 6–8 months. Unfortunately, some cancer patients die within this time period and have no chance to benefit from this costly screening setup.

The introduced model system of 'humanized mice' allows detailed analysis of additional sophisticated strategies to combat cancer. Immunotherapy, particularly, offers the promise of targeted therapy in conjunction with few toxic effects. This idea might seem contradictory to the reader as it was stated earlier that the absence of a functional immune system is a major drawback in these models. Indeed, mouse models are poor predictors in answering questions about the human immune system in cancer defense, because pronounced phenotypic and functional characteristics distinguish the human from the murine immune system [30]. However, an encouraging solution to this dilemma, and therefore second major advancement of xenograft models, was first presented in 2011 by Wege *et al.*, who developed a novel humanized tumor mouse model [31]. Based on the existing humanized mouse model harboring a maturated functional human immune system, developed upon $CD34^+$ cell transplantation, the researchers demonstrated that concurrent transplantation of human breast cancer cells yielded solid tumors or tumor-cell effusions 12 weeks after co-transplantation [32]. Despite the co-injection of MHC-mismatched tumor cells and human hematopoietic stem cells, no evidence of rejection was observed in this novel humanized tumor mouse system [31]. However, detection of $CD4^+$ memory T cells, expansion of NK cells, and production of perforin and IFNγ furnish convincing evidence that the observed activation of the human immune system is most likely triggered by the presence of human tumor cells [31]. It is worth noting that the human immune response in these mice was preserved without immunological elimination of tumor cells, which accurately recapitulates the situation in cancer patients.

In summary, preclinical assessment of immunemodulatory anticancer strategies including cytokine, antibody, and vaccination strategies are facilitated through this system. Regardless, the already established high-predictive value of 'avatar mice', additional engraftment of the patient's immune system, as well as orthotopic implantation of tumorgrafts in these models not only contributes to the accuracy, but also offers extended testing opportunities of single and combinatory treatment regimens. In summary, recent advances in procedural methods in rodent-based xenograft experiments fostered a significant improvement in reliability of these models.

Genetically engineered mouse models in cancer research

As outlined above, human malignancies are driven by genetic alterations. Thus, the primary goal of cancer researchers is to develop cancer models on the basis of these disease-defining lesions. This experimental approach offers several advantages in studying the underlying mechanisms of cancer biology: first, tumor growth is initiated orthotopically; second, the host organism has a functional immune system; and third, the surrounding tumor stroma and vasculature come from the same species [19]. All together, the characteristics of these autochthonous models often recapitulate their respective human counterparts. 'The origins of oncomice' extensively reviewed by Hanahan *et al.*, date back to the 1980s and illustrate the first independent approaches to the design of murine tumor models, wherein neoplastic growth sprouted heritably in various organs depending on dominant oncogenes [33]. Over the past 30 years, a step-wise process has led to the development of a powerful toolkit to specifically manipulate parts of the mouse genome. Particularly, two fundamental types of genetically engineered mouse models (GEMMs) have evolved: in transgenic mice the oncogene or dominant negative tumor suppressor is expressed from a non-physiological locus, whereas in endogenous GEMMs these functions are executed from their native promoters. Initially, adaptation of homologous recombination-mediated targeting resulted in 'knockout mice', which were depleted of a gene of interest. Despite the previously mentioned benefits of genetically modified models, results from knockout mice risk underestimating true biological mechanisms, because life-long adaptive signaling responses are most likely to occur. The use of non-mammalian DNA recombinase techniques, such as Cre/*LoxP*, and Flp/*FRT,* and the possibility to employ regulable promoters gave rise to the creation of murine models allowing gene mutations in a spatially and temporally controlled manner (reviewed in [34]). In line with this argument, the authors want to reemphasize that human tumors usually harbor several hundred mutations, although only a minority are assumed to be high-frequency instigating 'driver mutations' [6,35,36].

Development of more sophisticated murine cancer models harboring several transgenes is currently underway; however, the interpretation of such models is complicated by uncertainty about the interrelationship of the various transgenes and lesions. In addition, limited cancer modeling in GEMMs becomes evident regarding the sequential nature of genetic lesion introduction in human neoplastic malignancies. Adjacent to the abovementioned site-specific recombinase systems, a further technology for conditional mouse genome manipulation comprises the 'Tet-Off and Tet-On' system. In these models, the transcriptional activation or repression of the target gene is reversibly turned on or off in the presence of tetracycline [37]. Thus, serial mutational events could potentially be modeled employing both systems in the same transgenic mouse, but the current lack of precise and reoccurring cell targeting for transgene

modulation confines this possibility. However, the continuous discovery of additional site-specific recombinase systems – Dre/*Rox* [38], SCre/*SLoxP*, and VCre/*VLoxP* [39] – inaugurates novel experimental approaches to dissect the interplay of several mutated genes in a cancerous cell.

The 'ideal' preclinical model for novel antineoplastic compound development comprises five characteristics as described by Ocana *et al.* [40]:

- Validation of the target;
- Information about the mechanism of action of the tested drug;
- Identification of pharmacodynamic (PD) activity markers;
- Characterization of the toxicity profile;
- Identification of resistance mechanisms and how to bypass them.

Whereas xenografts and PDX only partially meet these criteria (Table 2), GEMMs provide a thorough evaluation of all five aspects. Particularly, the latter offer a powerful experimental platform in mining for biomarkers of disease progression or drug response, and allow further characterization of potential drug resistance mechanisms and subsequent means of 'second-line' therapies to overcome resistance.

The role of GEMMs in antineoplastic compound development has only emerged within the past decade, but is anticipated to gain significance as cumulating resistances to molecularly targeting drugs are observed. Although the 'first generation' of novel targeted drugs was nearly exclusively tested in the 'less optimal' xenograft models, prominent examples like imatinib (BCR–ABL fusion protein) [41], gefitinib and erlotinib (mutated or amplified EGF receptor) [42], or trastuzumab (HER2 overexpression) [43] successfully translated into the clinical setting. Rising incidences of resistance mechanisms in these cancer-driving lesions or other signaling pathways within the same tumor cell not only require the development of second-generation anticancer agents, but also sophisticated preclinical models to include the analysis of the tumor microenvironment as a potential resistance mediator against novel drug formulae. Despite the relatively short time span since GEMMs entered the preclinical drug evaluation arena, first examples of efficacious 'bench-to-bedside' advancements have been reported: dasatinib, a second-generation BCR–ABL tyrosine kinase inhibitor, induces higher and faster rates of complete response and improved progression-free survival in patients with newly diagnosed chronic myeloid leukemia [44,45]. Similar drug efficacy validations were recently shown in patients diagnosed with adenocarcinoma of the lung who received the second-generation irreversible tyrosine kinase inhibitor afatinib, which targets *HER2* and *EGFR* mutations, in combination with chemotherapy [46,47]. Although drug intervention studies in these transgenic models are putatively more reliable in predicting clinical outcome – because the underlying genetic drivers are similar to those in humans – attention needs to be drawn to potential constraints.

Even though introduced conditional mutagenesis methods allow spatial and temporal activation/

Table 2. Comparative characterization of available murine models employing the criteria of 'ideal preclinical models'.

Model	Validation of target†	Mechanism of action†	Identification of biomarker†	Evaluation of toxicity†	Identification of resistance mechanisms†	Evaluation of drug response
Conventional xenograft models	Only partially possible (unknown effect of additional genetic mutations)	Only partially possible (unknown effect of additional genetic mutations)	Not possible (transplant model, different species)	✓	Not possible (unknown effect of additional genetic mutations, tumor microenvironment of different species, no functional immune system)	Ectopic tumor implantation: simple (calipers) Orthotopic tumor implantation: advanced (noninvasive imaging)
PDX	✓	✓	Not possible (transplant model, different species)	✓	Only intratumorigenic resistance mechanisms can be assessed	Ectopic tumor implantation: simple (calipers) Orthotopic tumor implantation: advanced (noninvasive imaging)
GEMMs	✓	✓	✓	✓	✓	Noninvasive imaging

†Characteristics originallly described by Ocana *et al.* [40].
GEMMs: Genetically engineered mouse models; PDX: Patient-derived xenografts.

inactivation of genes with exogenous chemicals or viruses, this event often occurs within a large number of cells within the organ/tissue of interest. In the case of subsequent malignant growth in multiple organ/tissue sites, this 'stage' of murine neoplastic lesions is often referred to as being a correlate of 'advanced disease' in humans. By contrast, onset of malignant growth in humans is mediated by uncontrolled proliferation of a single cell, resulting in a localized primary tumor. Generally, 'advanced disease' in patients diagnosed with cancer indicates that the cancerous lesion has spread within or beyond a particular organ/tissue. Accordingly, the genetic composition of these lesions presumably differs from the primary tumor since the hallmark of metastatic spread usually entails enhanced aggressive proliferation. Whether the term 'advanced disease' faithfully describes similar phenomena in animal cancer models and humans remains ambiguous. Forthcoming solutions to this hurdle include either defined injection of site-specific recombinase in easily accessible organs and tissues in germline GEMMs to induce neoplastic growth surrounded by wild-type microenvironments, or switching to nongermline GEMMs (nGEMM) (reviewed in [48]). This animal model carries genetic modifications only in some of its somatic cells; however, the germline cells are spared. Malignant growth is generated through individual transplantation into each recipient mouse of the model. Although the reliability of this experimental approach largely depends on the investigator's skills, this method is suitable to generate significant cohorts of animals for high throughput translational drug testing. In comparison to traditionally used germline GEMMs, nGEMMs also provide another advantage in cancer therapeutic evaluation. The applicability of the laboratory mouse as a model organism is based on its invariable genetic makeup. Consequently, orthotopically arising tumors also consist of homogeneous cells. On the other hand, genetic heterogeneity is a hallmark of humans in general, and especially of human malignancies. Thus, transplantation models additionally offer rapid drug testing in various murine backgrounds.

Conditional GEMMs have an invaluable impact on today's cancer research; however, it is worth remembering that these models also have limitations discouraging their universal use. For example, the generation of novel autochthonous models and their validation usually takes several years. The latter is especially troublesome since the vast majority of rodent models have incomplete penetrance, which is associated with a nonsynchronous and often prolonged latency of tumor growth. Furthermore, most GEMMs notoriously fail to mimic metastatic spread. Understandably, these characteristics create logistical and financial barriers regarding the usefulness of GEMMs in preclinical therapeutic studies; however, as specified below, the natural history of their cognate human malignancies also varies significantly from patient to patient. In addition, appraisal of genetically tailored antineoplastics in GEMMs requires sophisticated imaging modalities such as PET, MRI, and/or CT to monitor tumor growth and regression at serial time points in live animals. In line with the authors' remarks on tumorgraft mouse models, especially the transplantable nGEMMs of cancer provide valuable systems for scrutinized preclinical drug evaluation.

Limitations of preclinical animal translational research

Nearly all available murine cancer models utilized for drug evaluation studies are afflicted with the lack of sufficient validation. Even though a complete analysis of reproducibility, limit of cancer detection, relevance of results and mechanistic basis of each model is highly desirable to produce reliable research results, such experiments might triple the temporal and financial requirements in generating transgenic mouse models. Consequently, cancer researchers are obliged to take into account aspects of external validity while designing preclinical drug intervention studies. This includes the use of several different mouse models, hypothesis-driven design and statistics, and the discrimination against 'proof-of-principle' experiments [49]. Special attention needs to be drawn to identify parameters and biomarkers with proven relevance in clinical settings, or at least those that can be tested in the clinic. The optimal timing of outcome assessment is also important to define carefully. The effect of improperly set end points is clearly illustrated by the following example: a commonly chosen end point in animal studies is lethality. However, mortality in animals is far more often based on poor intake of food and water. Thus, the establishment of less confounding end points is preferred. Singh and colleagues, who recently performed an in-depth series of preclinical case studies in two highly validated *Kras*-mutant GEMMs to determine whether these animal models actually predict human therapeutic response better than xenografts [16], also argue that most preclinical studies using conventional xenografts lack appropriate end points of therapeutic response or outcome [50]. Thus, a qualified outcome predictor seems to be progression-free survival.

Detailed appraisal of the overall architecture of a preclinical drug intervention study reveals that this construction is prone to a number of significant flaws. Further pursuing the notion of validity in preclinical studies raises the need for adequate criteria of internal validity. Ideally, rodent-based treatment studies follow the concept of randomization and blinded evaluation. The overall design requires sufficient sample size and statistics as

well as consideration of gender and genetic background. Moreover, assessment of the general health status of the mice should be noted as an important factor [49]. Even today we still lack solid proof of whether mouse models of human cancer fail to predict clinical outcome in human trials reasoned by insufficiently modeled age-related aspects. First of all, financial and temporal restraints are the driving forces behind why the vast majority of murine cancer models are designed to exhibit symptoms of disease as quickly as possible. In contrast, most human cancers mimicked by these models develop far less acutely and instead reflect features of chronic disease. By the same token, further discrepancies in model architecture become evident comparing the age at tumor onset in humans and mice. Whereas cancer is mostly a disease of the elderly in humans, tumor growth is induced at a young age (6–12 weeks) in mice. However, although these particular age-related facets were not addressed in the preclinical *in vivo* trial performed by Singh *et al.*, the therapeutic response rates closely mirrored the human clinical scenario [16]. These correlations appear plausible as cancer patients eligible to enroll in clinical trials are required to have a good performance status, as well as adequate organ function to minimize the chance of clinical deterioration. Whether age-associated considerations should be integrated into current animal cancer models remains to be known. Furthermore, because of the biased study population composition, acknowledgment of pre-existing commonly severe comorbidities in cancer patients is crucial to keep in mind when comparing the efficacy of anticancer compounds in preclinical/clinical study populations with general cancer patients.

In addition to the potential pitfalls in conducting a preclinical study, the authors would like to point out more incongruities frequently observed in animal research testing drug interventions. Currently, the cancer-killing potential of novel drug formulae is mostly assessed in treatment-naive tumors in animals. The consequent evaluation in Phase I and Phase II clinical trials, however, includes patients with pretreated malignancies. Owing to standard practice in clinical trial execution, the qualified patients have undergone and progressed under first, second, or even further therapies and as a result their tumors have become refractory. Accordingly, cancer patients involved in clinical trials commonly develop advanced metastatic disease in multiple organ sites. In comparison to the therapeutic management of localized primary tumors, single metastases or minimal residual microscopic disease, wide spread metastatic disease poses a much greater challenge to the clinician. Preclinical murine cancer models rarely pursue existence of significant metastatic growth prior to initiation of novel drug evaluation. The relevance of the above-mentioned inconsistencies is further underscored by the surprising discovery of several groups, which demonstrate that spontaneous metastases are responsive to therapies that initially had no effect on the primary tumor [51–53].

The most difficult challenge in planning a preclinical trial involves the establishment of the most accurate treatment regimen. As outlined earlier, significant differences in physiology and anatomy between mouse and man impede assumptions on effective dosing schedules. Unlike in Phase I clinical trials, PK and PD studies are rarely addressed in preclinical rodent-based analysis. Whereas PK studies reveal the organism-related effects on applied drugs and determine appropriate drug-delivery methods based on factors, such as absorption, distribution, metabolism and excretion, PD examinations serve to evaluate whether the drug alters its molecular target in cancerous and noncancerous tissue, and consequent cellular effects. Due to the striking species-specific differences, it appears reasonable that the predictive value of PK and PD studies remains questionable. Accordingly, most rodent-based drug efficacy studies employ unrealistic dosages and drug exposure durations inapplicable in the human setting [17,54]. Remarkably, the route of anticancer-drug administration is an additional confounding factor in PK and PD evaluation. Whereas two common routes of therapeutic application exist in humans: enteral (via the digestive tract) or parenteral (via intravenous injection), for the ease of application, drugs are usually injected into the lower body cavity (intraperitoneal) in mice.

Antineoplastic drug development in the era of genomics and molecularly targeted compounds demands alternative efficacy assessment methods to the formerly used empiric screening procedures validated for cytotoxic agents. Thus, in a position paper of the Methodology for the Development of Innovative Cancer Therapies Task Force [55], consisting of international academic and pharmaceutical leaders, the following recommendations can be found in terms of selecting the most promising targeted agents for subsequent clinical evaluation: preclinical *in vivo* data should address PK and PD to provide evidence of molecular target inhibition/modulation as well as drug concentrations that are required to achieve these cellular modifications. In addition, the target effect should be correlated with efficient reduction of tumor growth, and ideally, the minimal level and duration of targeted inhibition of this anticancer agent are analyzed [55]. As contemporary antineoplastic agent development has shifted to a biology-driven approach, Damia *et al.* suggested to adapt the steps of preclinical development depending on whether the compound had a specific or unknown target [56].

Traditionally, subsequent dosing in Phase I clinical trials is determined as one-tenth of the lethal dose in animals. It is worth mentioning, this approach was first developed when systemic cytotoxic drugs were

tested in humans and the classic sequence of agent evaluation followed three main phases. Given the toxic nature of the drugs under investigation, the primary goal of Phase I studies is to determine the maximum tolerated dose. The emergence of molecularly targeted compounds, however, failed to fit the traditional developmental pathway for antineoplastic drugs because these agents do not follow monotonically increasing dose–toxicity–efficacy curves, commonly observed for cytotoxic formulations, their administration schedules and routes are different, and/or the antitumor activity requires distinguished assessment modes [57]. Thus, novel approaches in preclinical and early clinical study design are required to accurately evaluate the biological efficacy of contemporary antineoplastic compounds.

Predicting clinical outcome using cancer mouse models: how successful are we today?

As previously mentioned, cancer is one of the major threats humankind is facing today. The lessons learned from the past decades of cancer research uncovered that combating cancer is a multiplex challenge, which can only be tackled in a multidisciplinary approach. Despite tremendous advances in technologies allowing detailed insights into the underlying genetic drivers of human cancers, rapid translation of targeted molecular therapies persists as a crucial bottleneck in modern antineoplastic management of human malignancies. A giant stride towards urgently needed accelerated, targeted oncology drug development was achieved through initiation, conduct and evaluation of multidisciplinary 'co-clinical' trials [14,58]. The conceptual basis of this novel approach comprises the parallel conduct of murine-based preclinical and human Phase I clinical trials. To obtain valuable, real-time information from preclinical *in vivo* studies to predict clinical outcome in genetically stratified tumor-bearing patients, exploitation of appropriate GEMMs faithfully recapitulating the human clinical scenario is the key element in this experimental setup. Of prime importance, the initial co-clinical trial successfully resulted in switching the fatal natural history of acute promyelocytic leukemia into a curable disease through identification of a suitable, targeted combination treatment approach [58]. How can synchronized co-clinical trial approaches eliminate the unmet need of accelerated anticancer-drug development? Inevitably prerequisites are multidisciplinary teams of preclinical and clinical cancer researchers as well as pharmaceutical companies to agree on aspects of design, quality control and transparency of the co-clinical trial. Particular attention should be placed on the murine part; to balance the limitations described for GEMMs, additional utilization of avatar mice could improve the overall predictive value of the animal study. Furthermore, adequate infrastructure to house and treat the mice in addition to sophisticated noninvasive imaging modalities are indispensable in conducting a co-clinical trial. Ideally, these novel 'mouse hospitals' would be further developed into real 'preclinical testing centers' by complementary infrastructure for comparative pathology and bioinformatics for data mining and analysis [58]. Finally, the process of optimizing rodent-based preclinical studies to predict clinical outcome in human counterparts should culminate in establishing multiple testing sites to account for increased external validity.

Although there is little doubt that the 'co-clinical' trial approach exemplified by the acute promyelocytic leukemia story could be applied to other cancer entities, it remains questionable whether the exceptional improvements in treatment modalities, and subsequent patient overall survival, can be repeated. Nardella *et al.* argue that the major requirements to accomplish this task are at hand: knowledge of cancer-associated genetic mutations, GEMMs of various cancer entities and a broad range of potential compounds for immediate evaluation [58]. However, the weakest link appears to be the particular GEMM. The authors pointed out earlier that there is a discrepancy in genetic mutations found in human cancers versus the amount of genetic alterations that can be modeled in GEMMs. In this context, it seems reasonable to highlight that the number of genetic lesions differs according to the cancer type: lung cancers and melanomas tend to have many mutations (occasionally more than 100,000) [35,59–61] – whereas others have only a few mutations – for example, medulloblastomas, testicular germ cell tumors, acute leukemias, and carcinoids [6,62]. However, the average amount of driver lesions was found to be fewer than 15 [5]. This low number of driver mutations in any individual tumor is believed to affect a finite number of signaling pathways, indicating extensive rewiring of signal transduction networks in cancer cells [5,36]. Thus, it remains elusive whether GEMMs of different cancer entities are equally powerful to predict clinical outcome. The authors' group recently accepted the challenge to perform a co-clinical study on the most deadly malignancy with which patients are threatened: non-small-cell lung cancer [14]. To this author's knowledge, this is the first report showing that exclusive stratification for *Kras*-driven lung cancer is insufficient to predict treatment response. The authors demonstrated that the concomitant loss of tumor suppressors significantly impacts the overall response rate. In particular, concurrent mutation of *Kras* and *Lkb1* procured primary resistance to the combination treatment under investigation – namely docetaxel and the MEK inhibitor selumetinib. These findings are clearly beneficial to further improve the concept of co-clinical trials. The results demonstrate that, first, this concept is valid to predict clinical outcome of cancer entities prone to harboring numerous genetic mutations, and second,

that informed treatment decisions in genetic complex tumor types rely on more than just one biological marker.

Conclusion

Approximately 13% of all deaths each year are caused by cancer [202]. In the developed world, invasive cancer makes up the greatest part of these deaths [63]. To address this urgent need for effective anticancer drugs, effective means for high-throughput compound screening, evaluation and rapid preclinical-to-clinical translation are required. Here, the milestones of rodent-based model development are presented, which greatly facilitate the achievement of this goal. The integrated approach of utilizing these sophisticated animal models to parallel human clinical trials results in accelerated and highly predictable anticancer drug development. Ultimately, co-clinical trials will represent a powerful weapon against cancer, through rapid identification of effective molecularly targeted antineoplastic therapies, and hopefully turn fatal advanced malignancies into a chronic disease.

Future perspective

Despite the tremendous advances in oncology drug development offered through implementation of co-clinical trials, species-specific differences will always remain an insurmountable obstacle. Since the primary factor of drug efficacy assessment changed its nature from toxic to molecularly targeted, the question remains whether noncancerous human tissues can withstand biological effective doses of novel antineoplastic compounds. Recent advances in tissue-specific reprogramming of patient-derived cell lines, as well as attempts to model connected organ functions on microchips could presumably fill this gap in anticancer drug development.

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Executive summary

Background

- Increasing numbers of patients diagnosed with cancer as well as consecutive rising numbers of cancer-associated deaths worldwide illustrate the great need for accelerated antineoplastic-drug development.
- The success rate in anticancer-agent development is as low as 5% because most potential compounds show insufficient clinical activity in Phase III trials.

Role of murine models in cancer research

- Despite sharing 99% of common genes, *Mus musculus* and *Homo sapiens* differ significantly in terms of physiology, anatomy, metabolism, biochemisty, pharmacokinetics and toxicokinetics.
- However, sophisticated genetically engineered mouse models (GEMMs) have been shown to accurately predict clinical response and resistance rates.

Benefits & limitations of cancer mouse models in therapeutic assessment

- Xenografts of injected murine or human cell lines are widely used to analyze initial potential antineoplastic activity although this model system is afflicted with a significant number of downsides.
- Recent advances in humanized xenograft mouse models ('avatar mice') potentially promise to increase the probability to predict clinical efficacy of novel anticancer formulae.
- The cotransplantation of human hematopoietic stem cells and tumor cells in immunocompromized mice improves the model system through addition of a functional human immune response.

GEMMs in cancer research

- Autochthonous mouse models faithfully recapitulate human cancers and are valid for novel anticancer-compound testing.
- GEMMs offer the advanced possibility to identify and evaluate biomarkers of disease progression or drug response and can further be employed to characterize mechanisms of resistance.

Limitations of preclinical animal translational research

- A major obstacle confounding adequate prediction of clinical outcome based on preclinical animal studies is potentially attributed to insufficient external and internal validity within the study design.
- Pharmacokinetic and pharmacodynamic analyses are essential for successful translation into the clinic, but rarely included in preclinical trials.
- The emergence of molecularly targeted cancer drugs requires re-evaluation whether the current drug response criteria are still applicable.

Predicting clinical outcome using cancer mouse models: how successful are we today?

- Co-clinical trials are a powerful mechanism to pursue high-throughput drug screening and rapid translation of effective anticancer drugs into the clinical setting.

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