Predictive In Vivo Models for Oncology

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Abstract

Experimental oncology research and preclinical drug development both substantially require specific, clinically relevant in vitro and in vivo tumor models. The increasing knowledge about the heterogeneity of cancer requested a substantial restructuring of the test systems for the different stages of development. To be able to cope with the complexity of the disease, larger panels of patient-derived tumor models have to be implemented and extensively characterized. Together with individual genetically engineered tumor models and supported by core functions for expression profiling and data analysis, an integrated discovery process has been generated for predictive and personalized drug development.

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Improved “humanized” mouse models should help to overcome current limitations given by xenogeneic barrier between humans and mice. Establishment of a functional human immune system and a corresponding human microenvironment in laboratory animals will strongly support further research.

Drug discovery, systems biology, and translational research are moving closer together to address all the new hallmarks of cancer, increase the success rate of drug development, and increase the predictive value of preclinical models.

Keywords
Mouse models · Patient-derived xenograft (PDX) · Preclinical oncology · Translational research

1 Introduction

Tumor biology research and preclinical drug discovery both depend heavily on specific in vivo disease models. Historically, basic research and drug characterization were based on a handful of preclinical tumor models from each indication. Given our current knowledge about tumor heterogeneity, we can now understand why results from studies with 2–3 lung cancer models could have been only lowly predictive for the clinical outcome and a risky development work was a burden to clinicians and patients.

Almost in parallel with the new millennium, processes have changed substantially. This has been driven by increasing costs for the clinical development in contrast to often disappointing improvements for the patients. Growing insight into the fundamental genetic basics of the disease through analysis of gene expression and mutations and the development of fascinating new technologies in genetic engineering and bioinformatics – key word systems biology – have provided the technical basis for this paradigm shift.

As consequence, primary pharmacology processes in preclinical cancer research have changed, and the elementary task is the establishment of the right model and access to appropriate tools for each step of the drug discovery process (as shown in Fig. 1). This also requires former single disciplines to work more and more together, forming a more and more integrated process of preclinical drug discovery.

2 Demands on Target Identification and Validation Models

Innovative technologies in target identification and validation have also changed the request on the disease models. Have been a small number of extensively characterized tumor cell cultures and mouse models the standard for many decades, the target-driven approaches now require models reflecting better the clinical situation. Genotype-dependent stratification of patient cohorts to predict efficacy

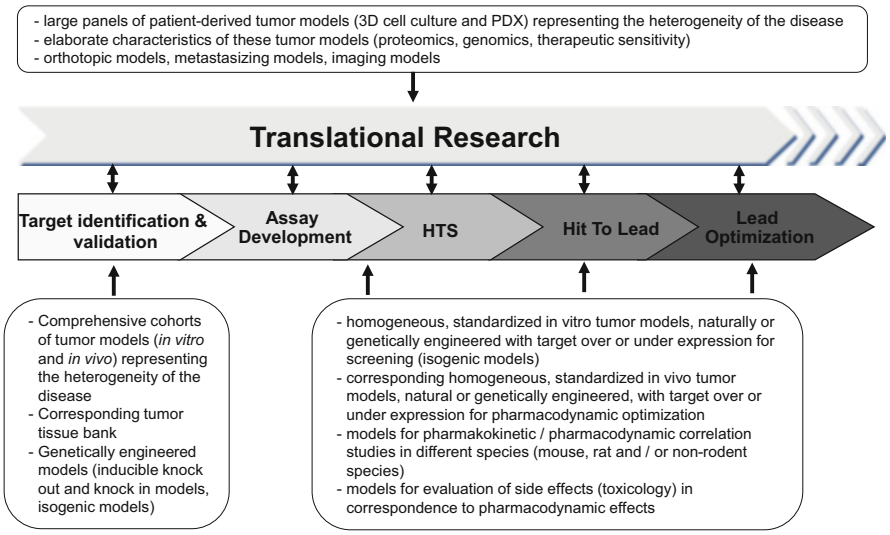

Fig. 1 Oncology drug development requires well-characterized panels of in vitro and in vivo tumor models, standardized analytical methods, as well as experimental settings to transfer bench-side targets into drugs for the clinic

of specific drugs is now seen as a prerequisite for the development of molecular-targeted therapies. Slamon and colleagues were the first using a cancer cell line panel to validate overexpression of Her2 as a predictive marker in breast cancer for the efficacy of Herceptin (Slamon and Pegram 2001). The lack of such studies in large cell culture panels in other indications has hindered the development of further epidermal growth factor receptor (EGFR) targeting drugs, i.e., gefitinib, cetuximab, or panitumumab. Initially, some unstratified studies failed, and only a posteriori genotype-dependent stratification of patient cohorts allowed predicting efficacy and further development of targeted therapeutics like cetuximab or panitumumab (Lièvre et al. 2006; Amado et al. 2008).

The requirements on new models for target identification and validation (TIV) include among others:

- Availability of large panels of tumor models (in vitro and in vivo) representing the heterogeneity of the disease
- Extensive data about the characteristics of these tumor models (gene and protein expression, gene amplifications, mutations, epigenetics, miRNA expression, histology, reference drug sensitivity)
- Corresponding databases containing all these information and tools allowing bioinformatic analyses
- Tumor tissue banks (frozen and paraffin-embedded tissue, tissue microarrays)
- Technology to generate genetically engineered models (inducible knockout and knock-in models, isogenic models)

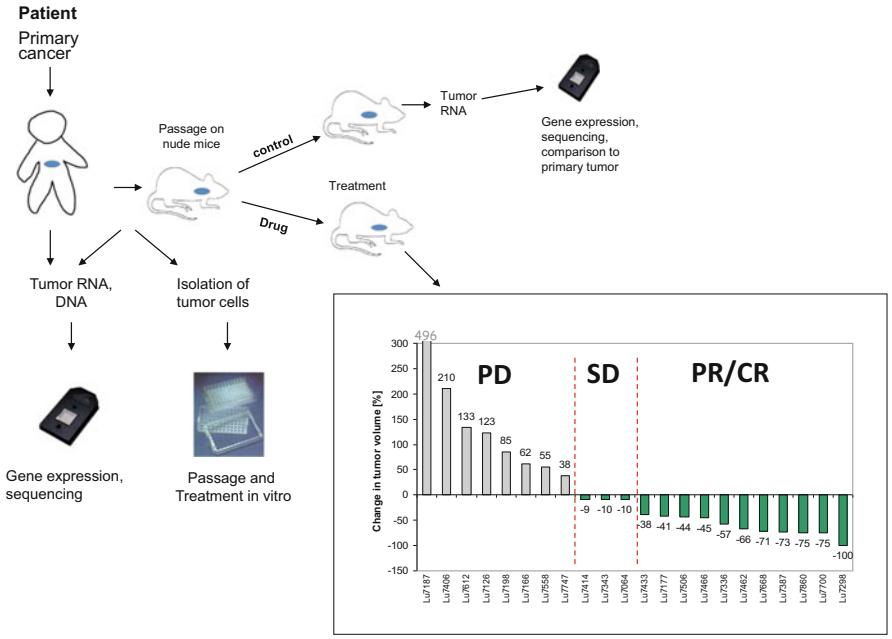

Fig. 2 Workflow of preclinical research using convenient PDX mouse models. Patient-derived material – expanded in mice – undergoes molecular biological and therapeutic screening. A waterfall plot distinguishes responder from nonresponder (Fichtner et al. 2008). RECIST criteria: *PD* progressive disease, *SD* stable disease, *PR*/*CR* partial or complete response

A key component of preclinical strategies is the so-called patient to mouse xenotransplantation model (PDX), established by transplantation of fresh patient material to immunodeficient mice (shown in Fig. 2). After a successful engraftment (growth to a tumor volume between 500 and 1,500 mm^3) within 2–6 months, the PDX can be used for serial transplantation over several generations (P2–P10). Expanded material can be used for drug response or biomarker studies, the establishment of cell lines, and molecular/histopathological analyses and is preservable due to cryoconservation (Scott et al. 2013). Target validation using a broad cohort of clinically relevant PDX models provides more reliable information. As FDA requests drug development to be accompanied by the development of a companion diagnostic test, both require close collaboration between the preclinical experts. Disease-related PDX panels are seen as the optimal basis for the detection of predictive, prognostic, and early-response biomarkers. Possible resistance mechanisms, predictors of response, and rational targets for combinations can be identified, and further the physiological mechanism of action can be analyzed (Amendt et al. 2014).

Next to elementary target or biomarker identification and validation, PDX models will serve as an important tool for the implementation of a personalized medicine.

3 Tumor Models in the Lead Identification and Optimization (LO) Process

The lead identification and optimization is more or less identical with the classical drug development process. Depending on the nature of the target, this will include in vitro assay development, followed by a screening phase of selected compound, peptide, antibody, or RNAi libraries to identify a lead structure. Once a lead structure has been identified, optimization processes are started, frequently in parallel for several leads (Fig. 3).

As the most difficult part of the targeted drug development, this part has to address the molecular mechanism of action in correlation to optimal pharmacodynamic activity (physiological mechanism of action), optimal pharmacokinetics (absorption-distribution-metabolism-excretion (ADME)), toxicity, as well as resistance development.

A large number of functions are getting involved in this integrated preclinical drug development to address:

- The extent of target inhibition in correlation to pharmacological effects (i.e., inhibition of tumor growth, blood flow, metabolism)
- Identification of main indications (primary tumors, metastases)
- Sensitivity on combination with other drugs (drug modifier screen, i.e., high-throughput (HTS) proliferation assays or siRNA technology)
- Sensitivity to drug transporters (ABC transporters), cellular uptake, and intracellular distribution

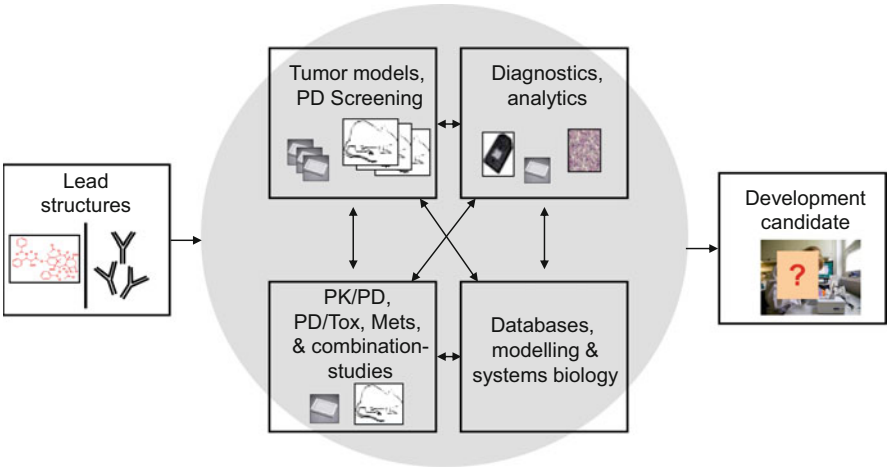

Fig. 3 Integrated research for novel drug candidates. Medicinal chemistry and experimental biology, supported by main preclinical functions, are interactively involved in the preclinical oncology drug development processes

- Gene regulation by the drug in sensitive and resistant models
- Mechanisms of apoptosis and effects on the immune system
- Potential adverse effects and their modulation
- PK/pharmacodynamics (PD) correlations and optimal treatment schedules
- Imaging of response

Similar to the drug development, the biomarker optimization might use or require some of the models in parallel.

In analogy to the TIV process, increased demands on the lead optimization have changed the requests on the disease models. The target-driven approaches now require models with defined levels of target expression which will be mainly generated by genetic modifications and cloning:

- Homogeneous, standardized in vitro tumor models, naturally or genetically engineered with target over- or underexpression for screening (isogenic models), models for classical drug resistance
- Homogeneous, standardized in vivo tumor models, natural or genetically engineered with target over- or underexpression for pharmacodynamic optimization (transgenic mice)
- Models for pharmacokinetic/pharmacodynamic correlation studies in different species (mouse, rat, and/or non-rodent species), models for evaluation of side effects (toxicology) in correspondence to pharmacodynamic effects

4 Translational Research (TR) Process

Translational research in oncology from the perspective of the drug developer should provide the simple answer: "who is the right patient for my new drug," whereas the oncologist is interested in: "which is the right drug for my patient." This means that in the later stages of cancer drug development and in the management of patients with cancer, "predictive biomarkers" are urgently needed which can be used to identify optimal target populations of patients; predict the efficacy of the drug and patient's response, resistance, and toxicity; and rapidly distinguish between nonresponders and patients who respond to therapeutic intervention (Kelloff and Sigman 2012). The major challenge for translational cancer research is the development of patient-specific models conserving the histology and genome of the donor tumors and providing the basis for experimental target validation, individual drug testing, and response prediction. Several cancer system biology consortia are currently developing new patient-derived xenograft (PDX) models. The PDX cultures maintained complex tissue architectures, intra-tumor heterogeneity, driver mutations, and marker expression. Screening of 57 PDX models with 12 compounds revealed pathway-specific drug responses (Rivera et al. 2014). These newly developed PDX models are providing efficient tools for personalized drug development under the given time constraints of clinical settings.

The US Food and Drug Administration (FDA)'s Center for Drug Evaluation and Research (CDER) has provided a guidance document on the qualification process for biomarker (titled "Draft Guidance for Industry: Qualification Process for Drug Development Tools"). Requirements set in this document make clear that the qualification process for a biomarker has many parallels to drug discovery and development, starting with biomarker identification and validation, followed by assay development and optimization, and finally followed by validation in clinical trials. In the preclinical oncology research departments from most pharmaceutical and biotech companies, the translational research has now become an integrative part of the development.

TR needs:

- Large panels of patient-derived tumor models (in vitro and in vivo) representing the heterogeneity of the disease
- Extensive data on the characteristics of these tumor models (gene and protein expression, gene amplifications, mutations, epigenetics, miRNA expression, histology, reference drug sensitivity, and corresponding databases containing all this information and tools allowing bioinformatic analyses)
- Orthotopic models, metastasizing models, and imaging models

This type of research is now frequently performed in academia-industry partnership.

5 Mouse and Rat Strains for Preclinical Oncology Research

Cancer research at bench side has been conducted by the use of animal models for decades. The transplantation of rodent tumor cells into laboratory mice or rats (syngeneic models) has been a widely spread approach for sensitivity testing, biomarker identification, and pharmacokinetic or metastasis studies from the early twentieth century till now. Some of the rodent tumors have been induced by exposure to carcinogens, which led to the development of several specific carcinogen-induced tumor models, i.e., the nitroso-methyl urea (NMU) rat breast cancer model (Shull 2007). For these models, a panel of mice or rat inbred strains have been developed (Table 1).

The observation that athymic nude mice have an impaired immune system, lacking functional T-cells, led to the development of xenotransplantation models. These models allowed for the first time the in vivo growth and passage of human tumors in a laboratory animal (Morton and Houghton 2007). The success of the nude mouse models has intensified the search for further immunodeficient mutations. The discovery of the SCID mutation in mice and crossbreeding with mice bearing the NOD or BEIGE mutation led to the development of further immunodeficient mice with T- and B-cell defects. These mice develop a severe immune deficiency and improved the xenotransplantations. These mice require

Table 1 Commonly used inbred mouse strains (Carter et al. 1952)

Mouse strain	Origin	Features
A	Strong, 1921	Breeding of Kalt-Spring-Harbor and BAGG albinos
BALB	Bagg, 1906	BAGG albino
C3H	Strong, 1920	Breeding of BAGG albino with DBA
C57BL	Little, 1921	Breeding of female 57 with male of Miß Lathrop
CFW	Webster, 1926	"Swiss" breeding of Rockefeller Institute
DBA	Little, 1909	Diluted brown; strains: DBA/1, DBA/2
SWR	Lynch, 1926	Swiss mouse

Table 2 Immune deficient mouse strains (Zhou et al. 2014)

Mouse strain	Features
Nude	T-cell^{-} B-cell^{+} NK^{+}
SCID	T-cell^{-} B-cell^{-} NK^{+}
NOD/SCID	T-cell^{-} B-cell^{-} NK$^{(+)}$, short lifespan
NSB	T-cell^{-} B-cell^{-} NK$^{(-/+)}$, short lifespan
NSG	T-cell^{-} B-cell^{-} NK$^{(-)}$, long lifespan, no human MHC or cytokines
NOG	T-cell^{-} B-cell^{-} NK$^{(-)}$, long lifespan, no human MHC or cytokines
RB	T-cell^{-} B-cell^{-} NK$^{(-)}$, long lifespan, no human MHC or cytokines

SCID severe combined immunodeficiency, *NOD* nonobese diabetic, *NK* natural killer cells, *MHC* major histocompatibility complex, *RB* H-2^{d} RAG2$^{-/-}$ IL-2rgc$^{-/-}$, *NOG* NOD-SCID-IL2rg$^{-/-\ (tm1Sug)}$, *NSB* NOD-SCID-B2m$^{-/-}$, *NSG* NOD-SCID-IL2rg$^{-/-(tm1Unc-J)}$

special standards for breeding and housing which cannot be discussed in this review (Bosma and Carroll 1991).

Actually, the need for more humanized mouse models led to the development of novel genetically modified mice optimized for xenogeneic investigations (summarized in Table 2) (Zhou et al. 2014).

Besides the humanization of mice, another proceeding has been the genetic intervention (knockdown or overexpression of certain genes) to generate transgenic rodents developing spontaneous tumors (Smith and Muller 2013) or using Sleeping Beauty (SB) transposon technology for insertional mutagenesis screening (Tschida et al. 2014). Genetically engineered mice (GEM) are substantial tools for drug development and preclinical "investigations" (Rappaport and Johnson 2014; Lee 2014). Despite the importance of such models for basic oncology research, we will focus on the prospects of patient-derived xenografts (PDX) and how the combination of patient tumor material and humanized mouse strains reaches almost completely the clinical situation.

Even if mice are the more customary laboratory animals, there are several studies using transgenic or transplanted rat models for drug sensitivity testing, biodistribution, or pharmacodynamic experiments providing comparably significant and excellent conclusions in cancer research issues (Lee et al. 2014; Tran et al. 2014; Cao et al. 2014; Zhang et al. 2013; McCullough et al. 2013). Although the genome of rats is identified and manipulable, they have the practical

disadvantage regarding cost and time economy due to their larger size and generation time when compared to mice. For some specific approaches (e.g., physiological/anatomic and hormone-related questions, experimental handling, need of organ material, and PK studies), the laboratory rat might be the better option.

6 Humanized Mice

A current challenge is to overcome the limitations of "classical" mouse xenograft models: the lack of a functional immune background as well as the replacement of the human neoplastic microenvironment by mouse stromal components. Tumor-stroma interactions and blockade of the immune response are well-known factors promoting the tumor growth (Goubran et al. 2014; Wang et al. 2014). The development of appropriate models for the preclinical evaluation of approaches targeting these mechanisms is the focus of tumor model developers. Especially cancer immunologists are limited in their research to immune competent mice with syngeneic tumor models. Given differences in the homology between human and murine proteins or pathways are making the development difficult and less predictive.

Recent approaches have been based on the cotransplantation of human cancer cells as well as human immune or stromal cells into an immunodeficient mouse. As an example, hematopoietic stem cells, engineered NK and T-cells, or mononuclear cells from the peripheral blood have been successfully engrafted on tumor-bearing NOD/SCID mice and used for the evaluation of T-cell-activating therapies (Zhou et al. 2014; Brischwein et al. 2006; Schlereth et al. 2005; Dreier et al. 2003; Fu et al. 2014; Wege et al. 2014; Thibaudeau et al. 2014; Rongvaux et al. 2014; Alcantar-Orozco et al. 2013). Several groups are working on the generation of humanized mouse models for oncological purposes. Fu et al. (2014) established a humanized ovarian tumor stroma due to the transplantation of normal ovarian tissues. The development of a human immune system after implantation of hematopoietic stem cells for a breast cancer xenograft model was described by Wege et al. (2014). The injection of $CD34^+$ cells into specific genetically modified mice results in the production of functional human monocytes, macrophages, and NK cells (Rongvaux et al. 2014).

In summary, xenotransplantation of patient tumors on "increasingly humanized mice" will strongly support predictive preclinical oncology research and moreover provide a fundamental basis for "personalized medicine."

7 Scopes of Patient-Derived Xenografts

Classical cell cultures lack the cellular interactions and structural properties of their donor tissues divesting spatial in vivo-like organization and intra-tumor heterogeneity. This frequently results in different gene expression profiles and drug response readouts (Cree et al. 2010). To better mimic the tumor's composition, in vivo PDX

models for various solid tumor entities have been established and studied with increasing intensity. Unsurprisingly, gene expression and proteome profiles of PDX differ considerably from their respective cell culture counterparts, as PDX rather resembles the genome of the donor tumor (Garralda et al. 2014; Monsma et al. 2012), as shown for ovarian- and colorectal cancer samples. This similarity allows an improved prediction of drug response in efficacy studies. The increase in predictive power prompted us to exploit PDX for accelerating early phase drug discovery. In the past years, preclinical studies using large panels of patient-derived xenograft (PDX) models grown in immunodeficient mice have demonstrated their predictive value for drug and biomarker development (Malaney et al. 2014). Although these PDX models are highly recognized as preclinical research tools, they require substantial resources.

Are preclinical models able to predict the results of clinical trials? Novel strategies to generate more predictive preclinical data for optimization of the clinical development are urgently needed. Appropriate animal models with close similarity to human biology can assure a higher predictability of preclinical studies.

In addition to the general question in which patient population of the drug should be tested (topic of the companion diagnostics development), three further questions have to be addressed by preclinical studies. Is the drug active in metastatic disease, in relapsed tumors, and in the frequency and mechanisms of treatment resistance (Decaudin 2011; Siolas and Hannon 2013)?

Although s.c. xenotransplanted tumors on mice have either a low metastatic potential or not sufficient time to metastasize as the fast growth of the primary tumor requires termination, several approaches have been developed to study metastasis. Most close the clinical metastasis process is modeled by orthotopic tumor transplantation, followed by either lymphogenic or hematogenic metastasis to the lymph nodes, lungs, liver, or peritoneum. Examples have been published for breast cancer (Wenzel et al. 2010), prostate cancer (Park et al. 2010), and lung cancer (Hoffmann et al. 2014). Evaluation of metastasis can be done either by counting visible organ metastases, evaluation of micrometastases by immunohistochemistry, or by human-specific PCR allowing detection of dormant tumor cells (Becker et al. 2002). This procedure is sometimes restricted by the fast growth of the primary tumor. The surgical removal of the primary tumor can permit a longer observation for tumor metastasis.

Given the mentioned limits to model the “natural” metastasis, several surrogate models have been developed. These approaches are using the intravenous, intracardial, or intraperitoneal injection of tumor cells to induce dissemination. This technology has been used successfully to model bone metastasis of breast cancer (Strube et al. 2009). As none of the known tumor models metastasize to the brain, we have developed a surrogate model by implanting either breast or lung tumor cells in the brain, simulating brain metastases (Hoffmann et al. 2009) and allowing the evaluation of drug activity.

Although many tumors initially respond well to the treatment, growth relapse is seen in the majority of patients. Preclinical information, whether a tumor would respond to re-treatment after initial response, is of utmost interest for the clinicians.

Using colon cancer PDX models, it has been recently demonstrated that this can also be simulated in this model system. While initial treatment resulted in tumor regression, a regrowth was observed shortly after treatment suspension. Further treatment cycles were able to re-induce tumor regression by a combination treatment, whereas the single treatments failed to demonstrate activity in recurrent tumors (Schmieder et al. 2014).

These methods can also be used to address the questions regarding resistance mechanisms. Development of second-line resistance to anticancer therapies can be induced due to sustained treatment over several generations of a xenografted tumor. The developed resistant tumors can be used to analyze mechanisms of resistance. Models for antiestrogen-resistant breast cancer have been developed, and by comparing gene expression of the parental and the resistant tumors, Her-2 upregulation has been identified as resistance mechanism (Sommer et al. 2003).

Human tumors accumulate genetic and molecular abnormalities, leading to broad heterogeneity. Large panels of molecular-defined and characterized PDX models reflecting tumor heterogeneity have increased impact for predicting the response to new therapeutic agents in the clinic. The reproducibility, renewability, and availability of tumor material are undisputed advantages.

8 Translational Preclinical Studies with PDX Can Identify Predictive Response Marker

Interesting data have been generated in a study using a large set of patient-derived NSCLC xenograft models (Fichtner et al. 2008). In this panel of NSCLC models, heterogeneous response to Sagopilone treatment was determined in an integrative preclinical phase II study (Hammer et al. 2010). Genome-wide gene expression analysis and mutation analysis of selected genes were used to identify potential markers of response and refractoriness and to explore the mechanism of Sagopilone's antitumor activity in vivo. Overexpression of marker genes (e.g., CA9, CA12, EPHA4, ITGA6) together with TP53 gene expression and mutation has been identified as potential predictive marker for response to Sagopilone (Hammer et al. 2010).

A large panel of colorectal cancer PDX models was developed and tested for drug sensitivity in parallel with a streamlined genetic characterization utilizing panel sequencing and gene expression. The study was used to evaluate to what extend PDX model-based technologies can support translational cancer research processes and even replace clinical experiments (Pechanska et al. 2013; Henderson et al. 2014). In this study it has been confirmed that kRas mutations are a strong predictor for resistance to cetuximab (with 86% specificity), and in addition mutations in bRaf and PI3K have been identified as additional predictive biomarker for drug response (Pechanska et al. 2013).

PDX of pancreatic cancer has been also used as a model for translational medicine (Behrens et al. 2014). For pancreatic cancer, similarity between the activity of gemcitabine in PDX models and respective clinical trial data is notable (Garrido-Laguna et al. 2011). Further, PDX can be utilized as a potential screening

platform for clinical trials as it could be shown in a prospective study that the combination of *nab*-paclitaxel and gemcitabine is effective in pancreatic PDX. This outcome is correlated with the clinical efficacy of the combination. Indeed, in a randomized phase III study, this regime has shown to provide a survival benefit for patients with advanced pancreatic cancer, and it is likely to become a standard of care in this setting (Von Hoff et al. 2013).

In another PDX study with gemcitabine, the expression of gemcitabine-activating enzyme deoxycytidine kinase was identified as a predictor of drug efficacy. A subsequent analysis of this marker in clinical samples confirmed these results (Rubio-Viqueira et al. 2006; Sebastiani et al. 2006).

9 Current Limitations

One disadvantage of PDX is the loss of the human tumor microenvironment during engraftment of patient material. This may affect tumor progression and is discussed as one reason for the low take rate of breast and prostate xenografts (Hidalgo et al. 2014). A review of Fang and DeClerk (2013) showed clearly the impact and benefit of the tumor microenvironment as target for anticancer treatment. Several integrin inhibitors (EMD 121974, CNTO 95, MEDI-522) that impair the communication between tumor cells and extracellular matrix are under clinical investigation (Dechantsreiter et al. 1999; O'Day et al. 2011; Hersey et al. 2010). The therapeutic potential of the tumor surrounding tissue is discussed for several entities, like pancreatic (Rossi et al. 2014), breast (Nwabo Kamdje et al. 2014), and prostate cancer (Chiarugi et al. 2014). However, after xenotransplantation, the human stromal components are replaced by a murine texture within 3–9 weeks (Hylander et al. 2013). With respect to therapeutic approaches targeting the human tumor microenvironment, the classical PDX models are therefore less feasible. The stroma replacement can be decelerated by the engraftment of large, non-disrupted tissue fragments and by the use of NOD/SCID mice with knockdown of IL2Ry (Bankert et al. 2001, 2011). In addition, the cotransplantation of human fibroblasts has been evaluated to generate PDX models with a more "humanized" microenvironment (Hoffmann unpublished results).

As mentioned earlier, a human microenvironment is strongly needed for another pillar of tumor therapy – the activation of immune reactions. Xenotransplanted human tumor cells are growing well in immunodeficient mice, very similar to the patient where they have escaped the body's immune control. Whereas in the patient a functional immune system is present and can be redirected against the tumor cells, the currently used immunodeficient mouse strains are mainly lacking functional immune cells (i.e., tumor-associated macrophages, dendritic cells, cytotoxic T-cells) and secretion of inflammatory cytokines (Fang and DeClerk 2013; Duechler et al. 2014; daChuna et al. 2014; Paulsson et al. 2014). Consequently, the preclinical evaluation of immunotherapeutic strategies in PDX has certain limitations. Therefore, the less predictive syngeneic mouse models or the cotransplantation of human peripheral blood mononuclear cells has been the

standard for the evaluation of immunological therapies. In the last years, transgenic mice stimulating the differentiation of cotransplanted human hematopoietic stem cells have been developed. These mice will develop a functional human immune system, allowing the analysis of new immune therapies (Alcantar-Orozco et al. 2013; Cook et al. 2013; Futakuchi and Singh 2013; Reisfeld 2013; Stromnes et al. 2014).

The establishment and maintenance of patient-derived xenografts are time and cost intensive. Depending on the tumor type, engraftment rate of P1-generation ranges from 15% to 80% and usually takes 2–3 months. The following conservation, characterization, and validation process need further 4–6 months setting the time lines between 6 and 9 months.

These time lines are challenging for the use of the PDX for individual drug response prediction studies. Data will not be available for first-line treatments; however, it could be of valuable help for planning second-line therapies after tumors relapsed.

Working with in vivo tumor models set high demands on the qualification of the scientific personal and the laboratories (clean room, biobanking equipment, and molecular biology).

10 Outlook

Depending on the stage of the drug discovery program, different models are required. For primary in vitro screening, cell lines can be utilized easily from the available large panels or generated by genetic engineering. They can be selected based on the target or the question to be answered. For secondary in vitro screening, larger panels of tumor cell lines with known sensitivity or resistance to available standard drugs are used for further profiling.

Classical 2D cell cultures lack the cellular interactions and structural properties of their donor tissues divesting spatial in vivo-like organization and intra-tumor heterogeneity. This frequently results in different gene expression profiles and drug response readouts. To better mimic the tumor's composition, in vitro 3D models for various solid tumor entities have been established and currently studied with increasing intensity.

Often a differential pattern of sensitivity can be observed using in vivo models. This gap between in vitro and in vivo activity constrains that in vivo experiments are still crucial and remain an integral part to evaluate tumor response in the near future.

Although mouse xenograft models derived from established human cancer cell lines have undoubtedly enhanced the understanding of the antitumor activity of novel anticancer agents, these models have several disadvantages. Depending on the number of cell passages, xenografts can behave very differently to the primary tumor (Haddad and Yee 2008), and combined with other deficiencies in preclinical approaches (Sharpless and Depinho 2006), this can reduce the relevance of established xenograft models for predicting the probability of success of anticancer

drugs in clinical studies for some tumor localizations. Analysis of antitumor activity in patient-derived xenograft (PDX) models has provided a more accurate selection process for the identification of agents which have activity in clinical trials, suggesting that some of these models may provide a useful hint for activity in the clinic (Furman et al. 1999). A fundamental move for the improvement of PDX is the humanization of these models. Different approaches such as the establishment of a human stroma, the cotransplantation of human hematopoietic stem cells, or the development of humanized homing niches have been successfully realized (Fu et al. 2014; Wege et al. 2014; Thibaudeau et al. 2014). Another effective method is the generation of novel mouse strains with humanized setting dropping highly informative preclinical data (Zhou et al. 2014; Rongvaux et al. 2014).

Genome-wide analyses of gene expression using oligonucleotide microarrays have allowed the determination of molecular characteristics present in xenograft models that mirror tumor behavior and relate to disease progression and survival (Nevins et al. 2003). Furthermore, correlations between the growth of xenograft models derived directly from patient tumors and the clinical prognosis of donor patients have been reported (Angevin et al. 1999; Peterson and Houghton 2004). In the future, the use of patient-derived human tumor xenografts will therefore play a key role in the search for more efficacious cancer treatments (Perez-Soler et al. 2006; Fichtner et al. 2004, 2008; Becker et al. 2004; Garber 2009). The ability to identify and assess antitumor activity in well-characterized xenografts in correlation with particular genetic or molecular characteristics may aid the development of new therapeutic regimens.

Conclusions from what we discussed here are:

- Drug discovery, systems biology, and translational research are moving together to address all the new hallmarks of cancer and increasing the success rate of drug discovery.
- In vitro versus in vivo models or vice versa – both models have limitations and advantages, however, when used critically, all generate important and reliable results.
- Panels of patient-derived xenograft (PDX) models represent an important tool for translational research.
- Predictive value of the preclinical models is increasing steadily; however, even genetically engineered “humanized” mice are still not men.

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