

Patient Derived Xenografts of Solid Tumors and Hematological Malignancies: Model Systems for Cancer Drug Development and Tumorbiology Research

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Published Date: December 19, 2015

ABSTRACT

In recent years, there have been multiple efforts in the establishment and characterization of large collections of Patient Derived Tumor Xenograft (**PDX**) models for cancer research. Although this model system was developed already in the late 1980's it came into focus lately due to its predictivity for clinical outcome and utility in biomarker development. PDX models mainly retain the histological and genetic characteristics of the donor tumor and remain stable across passages. They preserve cell-autonomous heterogeneity thereby representing very well the molecular landscape of the corresponding disease. Since triple immune deficient mouse strains like *NOD/Shi-scid/IL-2Ry^{null}* (NOG) or *NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ* (NSG) mice are made available to the scientific community; PDX of hematological malignancies could be established to a similar extend as their solid counterparts. Despite the incontrovertible advantages of PDX as preclinical models in drug development, pharma research faces a high failure rate combined with rising research and development costs of new drugs particularly in early clinical development. In view of success rates below 5% for innovative cancer drugs, improvements in model development are urgently needed. This book chapter summarizes the current state of the art in the field including technological issues, practical applications, challenges and shortcomings, and proposes some future directions, how to overcome the latter.

INTRODUCTION

Preclinical studies in Oncology are frequently based on the use of mouse models. On average, the protein-coding regions of mouse and human genomes are 85% identical and the mouse genome can be easily manipulated. However, the genomic identity between *H. sapiens* and *M. musculus* cannot compensate for significant species differences influencing the outcome of experiments investigating specific aspects of tumor biology or anticancer drug activity. Rodent tumor models have been generated since the 1960s, with xenografts of human tumor models emerging in the 1980s [1]. The development of the *nu/nu* mutant mouse lacking functional thymus tissue and therefore T-cells enabled the engraftment and serial transplantation of human tumor tissue into mice [2]. With the development of more immunosuppressive mouse strains like SCID, NOD/SCID or most recently NOD/SCID/IL2R γ null (NOG and NSG) mice the engraftment rate of human tumor tissue improved significantly over the last 30 years. Notably the latter enabled not only an improved engraftment of solid tumors but the establishment of PDX from hematological malignancies namely acute leukemia and different types of lymphomas [3,4]. Pioneer studies conducted in the 1980s already showed a high degree of correlation between clinical response and corresponding PDX sensitivity towards cytotoxic agents in Non-small cell lung cancer and childhood rhabdomyosarcomas [5,6]. In addition, PDX models have been used to conduct preclinical phase II studies with classic chemotherapeutics [1]. In our days, there have been multiple efforts in industry as well as academia to establish large panels of well characterized PDX models covering a wide range of different tumor types. Indeed, these collections are becoming the preferred research tool to endeavor the optimization of the drug development process at multiple steps in particular for target validation and translational studies [7-10]. Currently, these collections represent the preclinical oncology platform displaying most consistently the complexity of tumor heterogeneity and molecular diversity of human cancers.

PDX OF SOLID TUMORS

The methodology of establishment and propagation of solid tumor PDX has been described by multiple groups during the last decades [1,8,11-25]. The approach is very straightforward, starting with fresh surgical tissue, sectioning it into 4-5 mm³ pieces, followed by subcutaneous or orthotopic implantation into immune deficient mice (Figure 1). The amount of time required for tumor engraftment in solid tumors varies among different indications and is additionally influenced by the number of implanted viable cells and the recipient mouse strain. In general, it takes between two months and four months, although failure of engraftment should not be ascertained until at least a ten months observation period. Up to now there has not been a comprehensive comparison among different hosts, such as athymic nude mice (*nu/nu*), NOD/SCIDs, NOG or NSG affecting time-to-engraftment, take rate, genetics, or histology. Higher take rates are achieved with donor tumor tissue, from fast growing hormone independent tumors with a clinical unfavorable prognosis (e.g. pancreas carcinoma with a take rate of >75% [26]) compared

to slow growing hormone dependent tumors (e.g. prostate carcinoma with take rates below 10% [27,28]). Within one indication/entity it is reported that successful engraftment of respective patient tissue predicts a higher risk of tumor recurrence [29]. After three to four passages sufficient tumor material can be expanded for drug treatment experiments. Extensive molecular analyses can be performed on established PDX to characterize these models for predictive biomarker development (Figure 1) [30,31]. The determination of sensitivity against Standard of Care (**SoC**) treatment is maybe the most important characterization step for PDX as a tool for innovative drug development. Further characterization includes histological and immunohistochemical examination and more recently also proteomic based platforms [32]. Indispensable to such characterization studies is the ability to validate relevant specimens from larger cohorts at different time points and under different treatment regimens at an early stage for the clinical relevance of the potential biomarkers. One of the main advantages often noted with PDX models is the maintenance of the original tumor architecture and histological characteristics, particularly when compared to cell line derived xenografts [14]. The preservation of tumor heterogeneity is another advantage of PDX compared to (CDX). Projects such as The Cancer Genome Atlas have demonstrated the remarkable heterogeneity across tumors, previously believed to be of the same subtype [33]. Large collections of PDX models of a defined malignancy represent very well the molecular landscape of the corresponding disease. Furthermore, the understanding of the individual intra-tumoral heterogeneity within one patient led to a completely new understanding of the biology of the disease [34,35]. This cell-autonomous heterogeneity is very well reflected in PDX models [36]. However, intra-tumoral heterogeneity induced by the cross-talk of tumor cells with different compartments of the TME is only partly reflected in the PDX models. Despite the fact that tumor architecture is very well preserved the components of the Tumor Microenvironment (**TME**) are of murine origin. Tumor infiltrating lymphocytes are missing almost completely as the host is immune deficient. Due to described similarities between PDX and clinical samples of patients' tumors they are a useful model also in studies where the availability of a substantial amount of viable tumor material is a prerequisite such as flow cytometry based cell sorting or any kind of ex vivo studies like spheroid formation assays or tissue slices. Amongst other application PDX models are extremely helpful in cancer stem cell research, when treatment experiments render it possible to study the influence of conventional chemotherapy on the fraction of cancer stem cells [37,38].

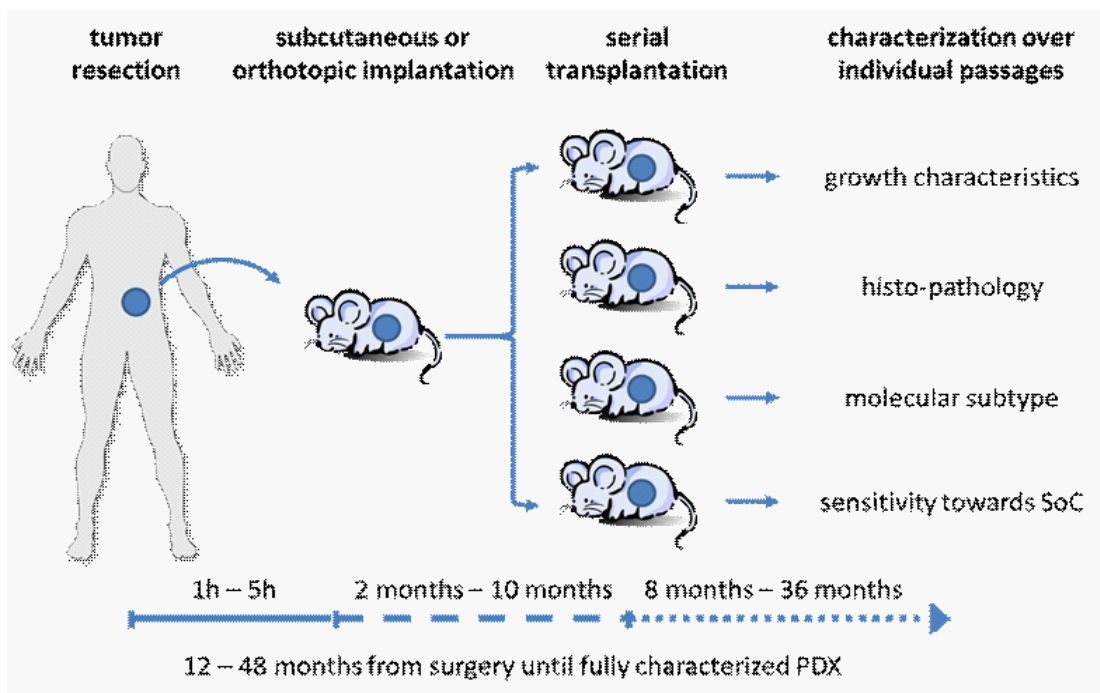


Figure 1: Development of patient derived tumor xenografts from donated tissue of cancer patients undergoing surgery. Human tumor tissue is resected during surgical intervention and implanted within a few hours either subcutaneously or orthotopically into immune compromised mice. The number of implanted mice in this first passage is restricted by the amount of available material. Within two – ten months the implanted tumor material starts to proliferate. Duration and extend of proliferation depends on multiple factors like viability of the implanted tissue and individual diagnosis and prognosis of the donor patient. Subsequent serial transplantation is performed in larger cohorts of mice and is meant to characterize the model in detail and to preserve PDX material for future experiments. Extensive molecular characterization and histo-pathological examination define the subtype of the established PDX. The determination of sensitivity towards Standard Of Care (**SoC**) drugs completes the characterization process and classifies the individual PDX model as a valuable tool for cancer drug development and tumorbiology research.

PDX OF HEMATOLOGICAL MALIGNANCIES

The development of SCID and NOD/SCID mice lacking functional T- and B- cells led to the possibility to engraft patient derived leukemic cells in mice. Reports mainly focused on stem cell research and other aspects of tumor biology, but not on the use of PDX as a drug development platform [39,40]. This was mainly due to the fact that the level of malignant cell engraftment in NOD/SCID mice is frequently low with typical engraftment levels of 0.1–5% of the mouse bone marrow and no possibility to maintain the individual model by serial transplantation. In addition,

prolonged engraftment of leukemic cells in NOD/SCID mice was limited by the development of spontaneous thymic lymphomas and a reduced life span. New strains, also called the new gold standard of immunocompromised mice, with a targeted deletion in the IL-2 receptor common γ -chain, based on NOD/SCID mice (NOD/LtSz-scid IL2R γ c null ,NSG and NOG) resulting in the severe impairment of B-,T- and NK-cell function, provided an improved environment for growth and development of human normal and malignant hematopoietic cells. NSG or NOG mice also have an improved life span of >90 weeks and are not prone to develop thymomas in contrast to NOD/SCID and SCID. The engraftment and propagation methodology differs slightly from the PDX of solid tumors. PBMCs or bone marrow samples from leukemia patients are injected either in the blood stream (intravenously, i.v.) [41] or into the bone marrow (intratibially, i.t.) [42] of recipient mice. Serial transplantation is also performed via i.v. or i.t. injection of the human cells harvested from spleen and bone marrow from donor mice. Validation of the models is done in a similar way as described above for the PDX of solid tumors. Take rates for acute malignancies like AML or ALL are around 35%, the time, until engraftment could be determined, was reported between three to six months [4,43]. Based on data of our own group “watch-and-wait” for up to 12 months after tumor cell injection, enhanced the take rate of these models significantly. Tumor load is usually determined via overall survival and detection of human leukemic cells in the peripheral blood of the murine host by flow cytometry. When animals have to be sacrificed, detailed analyses are performed of the infiltrated hematopoietic organs like spleen, bone marrow, liver and peripheral blood by flow cytometry, immunohistochemistry and FISH. These data not only indicate the exact engraftment rate and localization, but also the clonal heterogeneity of the engrafted leukemic cells. Recent data from our group, as well as others, suggest that serial expansion does not severely alter molecular sample characteristics of AML cells. Thus, PDX models closely resemble the disease they originate from [4]. Nevertheless, it has to be noted that other groups report of clonal selection during the engraftment process [44]. A very convenient and reliable read-out for tumor load determination is available using optical imaging methods. For this purpose cells have to be transfected with the Luciferase gene, thereby enabling their detection in the living mouse by Bioluminescence Imaging (BLI) [4,45,46]. Despite the incontrovertible advantages of BLI the indispensable *in vitro* transfection step is likely to influence the molecular make-up of the cells including their genetic heterogeneity. Another important observation is the correlation between engraftment capacity of cells of different acute leukemia and the prognosis of the donor patient. The ability of cells derived of acute leukemia to engraft in immune suppressed mice seems to be an inherent property of the malignant cells, independent of any pretreatment, cell frequency or source, which is directly related to prognosis [47,48].

CURRENT CHALLENGES

Human tumor xenografts in immune deficient mice have led to valuable insights into the biology of human cancer. Nevertheless, industry and academic pharma research faces a “productivity gap” with rising research and development costs combined with high failure

rates of new drugs, particularly in early clinical development [49-51]. This lack of translational capability may originate from the fact that in PDX the human non-malignant cells summarized as TME are replaced within a few days by their murine counterpart [52]. The great potential of studying the biological behavior and effects of anti-cancer therapies in many different human tumor xenograft models in-vivo is mainly limited by the fact that human cancer cells interact with a tumor microenvironment composed of murine cells. In comparison with the real situation of a human tumor growing at the site of origin, the interaction with murine cells in the respective organ system ("orthotopic xenograft models"), or in the subcutaneous compartment ("ectopic xenograft models") has been shown to affect tumor growth, invasion and metastasis as well as the composition of the stem cell compartment [53] and mechanism of resistance [54]. The corresponding limitations and potential pitfalls of murine xenograft models have been recognized and described extensively [13,55-57]. Given that tumor cells communicate with non-malignant cells in the microenvironment in a complicated, bi-directional crosstalk [58], it is obvious that animal models of cancer should reflect the outcome of this interaction when intended to evaluate its impact on the response of the tumor cells to different anti-cancer agents.

ADVANCED PDX

In recent years, a much better understanding of the pivotal role of the TME for cancer biology has led to the development of new drug targets that modulate the TME in order to support more effective tumor cell killing or to evoke an anti-tumor immune response [58,59]. To overcome current limitations of PDX models, parts of the murine TME can be eliminated and supplemented with immune cells and stromal components of human origin. As the TME is made up of multiple components, it is at least technically challenging to reproduce it in a living animal. To begin with, Extracellular Matrix (**ECM**), is known to alter gene expression, differentiation and stem cell features of tumor cells [60,61]. Matrigel, as a substitute for ECM, is widely used and known to enhance engraftment capacity in PDX. Nevertheless, it is of murine origin and suitable synthetic human alternatives are available and should be considered as biological more relevant alternatives. Furthermore, as ECM structure is tissue specific, researchers should consider the use of orthotopic transplantations where possible [36]. Irrespective of the indication, subcutaneous implanted PDX do not metastasize and rarely show infiltrative tumor growth [62]. In contrast, the same tumor model exhibits clinical relevant metastasis pattern when implanted orthotopically [63]. Due to this fact orthotopic PDX develop complications relevant to the clinic, which are not observed in subcutaneous models. The individual metastatic pattern of a PDX has close resemblance with the donor patient's [64]. Up to now there is no literature available comparing engraftment rates of subcutaneous vs orthotopically implanted PDX of the same patient cohort. However, entities, depicting very low success rates in the subcutaneous setting, are reported with higher engraftment rates in an orthotopic approach. This primarily refers to hormone dependent tumors like breast and prostate cancer [65,66]. Studies comparing drug sensitivity profiles of subcutaneous vs orthotopically implanted xenografts, revealed significant differences

indicating the importance of the TME for drug sensitivity [67]. As a matter of fact, the read-out in an orthotopic setting is more sophisticated as in a subcutaneous growing xenograft. Furthermore, the required surgical intervention is clearly more challenging. However, the recent development of new magnetic resonance imaging and micro-imaging techniques may minimize at least the issue of tumor load determination.

The influence of Cancer Associated Fibroblasts (**CAF**) on drug resistance, tumor growth behavior, including metastasis, and tumor heterogeneity is extensively described [68-73]. Due to this pivotal role many researchers start to co-inject fibroblasts of different sources to supplement the classical PDX model [74]. Beside the technical challenge that non-malignant cells do not engraft reliably for more than a few weeks in immune compromised mice, the impact of non-matched co-injection of fibroblasts and tumor cells is up to now not completely elucidated. To circumvent the latter, co-engraftment of matched stromal components should be considered wherever possible. Such an approach would significantly increase the advantages conventional PDX already have. Patient-derived fibroblasts can be relatively easily isolated and expanded *in vitro*. Thus, there is extensive literature available about new, sophisticated *in vitro* systems co-culturing matching and non-matching CAFs, tumor cells and additional parts of the TME, leading to more predictive preclinical data sets [75]. The translation into the *in vivo* setting is clearly lacking behind. Nevertheless, there are encouraging data about co-injection of tumor and stromal cells in different indications. In breast cancer, PDX fibroblasts are reported to promote invasion and metastasis as well as the clinical phenotype of inflammatory breast cancer, which is normally lost in murine models [76,77]. A more pronounced metastatic behavior as well as angiogenesis was determined, when human pancreatic tumor cells were co-injected with non-matched pancreatic stellate cells [78]. Increasing evidence from *in vitro* data indicate that CAFs mediate resistance to chemotherapy. CAFs can affect the sensitivity of pancreatic carcinoma cells to first line treatment gemcitabine *in vitro* [79]. Elimination of CAFs in a syngeneic mouse model of cancer improved markedly the effects of doxorubicin [80]. These and other data suggest that CAFs have a major impact on therapeutic resistance [81]. In the end, modelling tumor-stroma crosstalk *in vivo* is extremely complicated as the co-injection of stromal cells results in a chimeric tissue composed of human tumor and stromal cells as well as murine fibroblasts. Up to now, it is neither possible to deplete murine stromal cells from PDX tissue nor to prevent the infiltration of murine fibroblasts from the surrounding tissue into the tumor bulk. Controlling the degree of chimerism and a deep understanding of the bi-directional crosstalk between all involved cell types is an indispensable requirement for the meaningful application of such *in vivo* co-culture models.

With regard of the inevitable basic role of the immune system in tumor biology, the necessity to use immune compromised mice might be the most obvious drawback of PDX models. More than ever in the light of the growing importance of immune modulatory approaches in cancer drug development. Tumor cells are broadly thought to be antigenic due to their multiple mutations in coding exons resulting in a phenotypic make-up reasonably different from the normal cells

they evolved [82]. However, most tumors eventually progress and evade the immune surveillance by inducing immune tolerance. The pro-inflammatory microenvironment in the tumor tissue established by CD8⁺ T-cells, Tumor-Associated Macrophages (**TAM**), NK-cells, and others leads to an immune suppressive signaling cascade enabling the tumor to evade immune surveillance. The ability to influence the immune system is often related to prognosis: the cytotoxic to regulatory T-cell ratio is a strong prognostic marker in many solid tumors [83]. The fact that macrophage and CD4⁺ T-cell recruitment following intensive chemotherapy e.g. in breast cancer patients is associated with significantly reduced recurrence-free survival, follows the same line [84]. Clearly, the co-culture of patient immune cells and patient derived tumors in an *in vivo* setting would trigger the study of novel therapies targeting tumor-immune interactions and in parallel allow for novel insights into tumor biology. Mice supplemented with human immune cells, so called “humanized mice” should meet at least three criteria: (a) All lineages and subsets of human hematopoietic cells should develop in the mouse host, and they should be maintained in proportions and localizations similar to those observed in healthy humans; (b) these cells should be functional, i.e., capable of mounting innate and adaptive immune responses *in vivo*; (c) they should allow for testing of therapeutic interventions and faithfully predict the outcome in clinical settings [85]. Two basic approaches are described for generating humanized mice: either engraftment is performed with Hematopoietic Stem Cells (**HSC**) from different sources or mice are injected with human Peripheral Blood Mononuclear Cells (**PBMC**). In the latter engrafted human T-lymphocytes show xeno-reactivity against foreign Major Histocompatibility (**MHC**) class I and II and other antigens of murine origin. As a result, T-lymphocytes cause an inflammatory infiltrate in different organs that leads to wasting and death of the animals after several weeks, a process known as Xenograft-Versus-Host Disease (**xGVHD**) [86]. The co-transplantation of human hematopoietic stem cells together with cancer cells seems to enable the co-existence of MHC-mismatched cells without inducing rejection [87]. Up to now treatment experiments are mainly performed using PBMC-humanized mice. These experiments give valuable insights into the possible benefit new immune modulatory compounds could bring to cancer patients as long as the influence of xGVHD on tumor growth is taken into account [88]. The implication of all these *in vivo* co-culture approaches is the combination of human tumor and stromal cells in humanized mice. The technical hurdles seem to be very high and it is more than challenging to build up such an *in vivo* platform with a degree of robustness, turnaround time and reproducibility needed in preclinical drug discovery. Nevertheless, the possible increase in effectiveness of the drug development process would be incontrovertible.

CONCLUSION

The establishment of PDX models that recapitulate the complexity and genetic heterogeneity of human cancer is an invaluable research tool enabling insights into tumor biology and strategies for developing new innovative approaches for anticancer treatment. A repository of extensively characterized PDX models of different entities can be used for drug screening and discovery,

tumor biology studies as well as biomarker development. Beside the discussed assets and drawbacks of the different PDX models some basic requirements have to be fulfilled. The quality of animal research has a major impact on the translational value of the PDX based experiments. Quality in this context begins at the animal facility, which should meet internationally accepted standards provided e.g. by Association for Assessment and Accreditation of Laboratory Animal Care (**AALAC**). Another critical point is the read-out and interpretation of the data. In many research groups, the design of animal studies, the experimental execution, and the evaluation of the data are under the purview of one, non-masked, person. Several studies elucidated that certain weaknesses, including this lack of masking, limit their translational value to human application [56]. Moreover, rigorous criteria have to be implemented to define anti-tumoral activity. Repeated measures of tumor burden by caliper are common practice irrespective of the investigated tumor type or study context. These simplistic criteria often used in mouse models do not match the challenging criteria of clinical or pathological investigations. These obvious discrepancies unfortunately contribute to the conflicting opinions about the relevance of mouse models as they impact directly their predictivity for subsequent clinical investigations [51]. Thus, additional metrics, e.g. histopathological examination and overall survival, have to be implemented. More than ever, in the context of evaluating the activity of immune-modulatory drugs, which primarily target immune cells rather than tumor cells. Overall survival provides a well-defined endpoint for the determination of treatment efficacy. Of note, survival must be followed after treatment to assess the complete life expectancy until tumor regrowth occurs. Despite a delay in tumor growth throughout the treatment period, a rebound effect after treatment can occur and is indicative of lower overall efficacy. Notwithstanding the technical challenge, labor and cost intensity of such detailed examinations; the translational value of PDX models is improved by putting multiple rigorous endpoints into effect. Beyond that interpretation of data has to be performed in dependence on clinical criteria. This implies that preclinical response rates should meet the Response Evaluation Criteria in Solid Tumors (**RECIST**) criteria which are based on size changes of the target lesion [89]. It should be noted that a chronic slowness of tumor growth rate can be considered a relevant outcome as compared with tumor regression if the intended goal is tumor growth delay [51]. On the other hand, despite all innovative models and read-outs the subcutaneously implanted patient derived tumor xenograft remains the core area of preclinical *in vivo* models because it is easy to follow and it has yet to be shown that more sophisticated tumor models would discover drugs missed by this gold standard. In conclusion, PDX models are an important part of the preclinical tool box being complementary to other preclinical oncology models, such as genetically engineered mouse models or cell line derived xenografts. Although establishment, characterization and validation of PDX models has been in existence for decades, their value in oncology drug development and tumor biology research is just becoming more and more evident. The different approaches to improve the PDX platform towards an even more clinically relevant model will secure the future of PDX research.

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