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Patient-Derived Xenografts as a Model System for Radiation Research

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Abstract

The cancer literature is filled with promising preclinical studies demonstrating impressive efficacy for new therapeutics, yet translation of these approaches into clinical successes has been rare, indicating that current methods used to predict efficacy are sub-optimal. The most likely reason for the limitation of these studies is the disconnect between preclinical models and cancers treated in the clinic. Specifically, most preclinical models are poor representations of human disease. Immortalized cancer cell lines that dominate the cancer literature may be, in a sense, “paper tigers” that have been selected by decades of culture to be artificially driven by highly targetable proteins. Thus, although effective in treating these cell lines either *in vitro* or as artificial tumors transplanted from culture into experimental animals as xenografts, the identified therapies will likely underperform in a clinical setting. This inherent limitation not only applies to drug testing, but also to experiments with radiation therapy. Indeed, traditional radiobiology methods rely on monolayer culture systems, with emphasis on colony formation and DNA damage assessment that may have limited clinical translation. As such, there has been keen interest in developing tumor explant systems in which patient tumors are directly transplanted into, and solely maintained *in vivo*, using immunocompromised mice. These so-called Patient-Derived Xenografts (PDX) represent a robust model system that has been garnering support in academia and industry as a superior preclinical approach to drug testing. Likewise, PDX models have the potential to improve radiation research. In this review, we describe how PDX models are currently being used for both drug and radiation testing and how they can be incorporated into a translational research program.

Keywords

patient-derived xenografts; kinomics; radiosensitivity; MicroTumor; tumor avatar; tumor proband

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Introduction

Clearly, one of the biggest challenges in the development of new treatments for aggressive solid tumors is that testable preclinical models do not faithfully represent the clinical entity very well and thus fail to predict success in the clinic.¹⁻³ Even with advanced target and drug discovery strategies⁴ that allow for the rapid discovery of new molecularly-targeted therapeutics for cancer treatment, it is often unpredictable which drugs will be effective for which patients. Indeed, for solid tumors, such as *Glioblastoma multiforme* (GBM) and renal cell cancer, there is an impressive amount of heterogeneity, even within the same tumor.^{5,6}

Recent research endeavors have undertaken the task to comprehensively characterize 20 different cancers at various molecular levels as part of The Cancer Genome Atlas (TCGA; <http://cancergenome.nih.gov>). The first cancer to be investigated by TCGA was GBM that serves as a prototype for the promise and the challenge of developing novel therapeutic approaches in cancer. While the TCGA research made great strides in discovering important genomic changes underlying GBM pathogenesis,^{7,8} susceptibility,^{9,10} defining GBM subtypes,^{11,12} and discovery of important molecular mechanisms underlying tumor transformation,^{13,14} it still remains to translate this knowledge into effective therapeutic strategies that can be used in the clinic.

Moreover, it has become increasingly clear that surrounding stroma and vasculature actively participate in the tumor phenotype. As such, models that rely solely on highly proliferative cells in standard cell culture, or even as subcutaneous xenografts, will often underperform when used for drug testing because these models severely underestimate the complexity of actual tumors in patients.^{2,3,15} This is no less true for the testing of radiation modifiers than it is for other therapeutic agents. Indeed, traditional radiation biology approaches including the colony formation assay (clonogenic assay) have not consistently translated to the clinic.¹⁶ Therefore, it is imperative that: a) patient-relevant molecular characteristics, and b) more realistic models of disease be incorporated into the drug discovery and development process in order to improve the dismal success rate of current approaches. The focus of our review is the use of Patient-Derived Xenografts (PDX) for radiation biology investigation. Herein, we discuss the general advantages of PDX as well as the challenges that this model system presents. We specifically discuss how PDX models have been used in the setting of radiation biology and how they may improve upon the rate of success in translating laboratory discoveries into clinical therapeutics.

Patient-Derived Xenograft Models

Cell lines are very difficult to derive from most patients' tumors, and those that are established develop in an artificially normoxic, glucose- and growth factor-rich environment that selects for a rapidly-growing and nearly clonal subpopulation from within the heterogeneous starting tumor preparation.^{2,15,17} These cells are less reflective of the true heterogeneity of a patient tumor, and testing therapeutics *in vitro* ignores many important aspects of tumor biology such as hypoxia and nutrient deprivation (bioenergetic stress), tumor/stromal cell/extracellular matrix interactions, and angiogenesis (highly influenced by the tumor microenvironment). In contrast, testing therapeutics of a patient's tumor growing as a mouse-hosted PDX is likely more reflective of the original complex tumor biology and

may more accurately predict successful translation to the clinic.^{2,15,18,19} The reason this model is not commonly used is likely due to the need for a specialized infrastructure, including approved and regulated animal facilities coupled with staff experienced with tumor processing and implantation, a process that is especially resource-intensive and expensive at the initial programmatic set-up (**Fig 1**).²⁰

Gaining meaningful insight into the response to therapy for many cancers is hampered by the significant heterogeneity at every level among tumors, even within patients' tumors of the same histopathologic type. Clinical trials generally focus on a single therapeutic agent although only a small percentage of tumors may be driven by the gene or protein that constitutes the therapeutic target.²¹ Requiring expression of the target before entry into a trial affords a useful criteria, but detected expression may not always correlate with the target being a driver of growth in a particular tumor.²² These factors reduce the likelihood of a favorable response, potentially underestimating the utility of a drug that may have benefit in a subset of patients. The enormous cost of drug development is compounded by this clinical inefficiency, in that development of a targeted therapy wastes limited resources expended in a clinical trial that may be falsely declared ineffective. By the same token, the homogeneity of established cell lines that have been selected by artificial growth conditions may suggest the potential efficacy of a specific agent that, upon clinical testing, proves to be ineffective to marginally effective since the "targets" in the cell lines are likely present in only a small percentage of patient tumors.

Patient-derived xenografts (PDX), which we typically refer to as "xenolines" to distinguish patient-derived xenograft tissue from xenografts established using immortalized cell lines, are a model system with the potential to overcome some of the limitations of studying cancer in immortalized cell lines.

Advantages of PDX over Traditional Cell Line Models

Xenotransplantation, the process of transplanting cells/tissues to another species, is not a new concept. Indeed, immunocompromised mice, such as athymic nude (*nu/nu*) mice, were used for xenografting human tumor cells over 45 years ago.²³ Immortalized cell lines have very high take rates in nude mice while direct transplantation of patient tumor tissue, including PDX, has a much lower take rate. However, the generation of other immunodeficient mouse strains (such as severe combined immunodeficient [SCID], nonobese diabetic/SCID [NOD/SCID], RAG-1 null etc.) has enabled an ever-increasing number of human tumors to be directly transplanted into mice.^{2,24,25} However, increasing immunodeficiency comes at a cost. First, severely immunocompromised mice are expensive to produce and maintain, which can preclude the use of these mice in drug screening studies. Second, in the case of experiments with radiation therapy, many of these models (outside of athymic nude) are inherently radiosensitive due to deficiencies in DNA repair such that radiation effects on host stroma and tumor microenvironment are amplified.²⁶ Although this can affect xenograft growth delay endpoints following radiation therapy, altering the radiation sensitivity of the stroma does not impact local control following radiation therapy.²⁷ In addition, there is a murine "drift" or "tumor transformation" that occurs especially in the severely immunocompromised mice in which the human tumors become

more mouse-like over serial passaging.^{20,28} For this reason, we prefer to use athymic nude mice for PDX development, maintenance, and experimentation. As glioma was the prototype tumor type for The Cancer Genome Atlas (TCGA), which is a comprehensive effort to understand cancer at the genetic level, the use of glioma PDX for cancer research remains a prototypical system, including for radiation research.

Our collective observations indicate that genetic changes commonly associated with high-grade glioma tissues are conserved and not amplified or substantially altered in xenografts, as compared with profound differences reported in cultured human glioma cell lines.²⁹⁻³² Tumor cells routinely established in tissue culture enjoy relatively high oxygen tension (20.8% vs. 5-10% *in vivo*), high levels of glucose (4.5g/L vs ~0.8-1.1 g/L plasma), and significant amounts of stimulatory growth factors (PDGF, EGF, TGF β , FGF) in serum-(a wound fluid)-containing media which is in stark contrast to the environmental pressures faced by tumor cells trying to grow in a living host. Under these conditions *in vitro* cultured tumor cells are strongly selected for rapid growth, thus amplifying genetic alterations that lead to cell proliferation. In contrast, glioma PDX grow slowly in mice, providing ample opportunity for tumor cell invasion of normal brain and the establishment of vascularization. These PDX tumors better model the genetic heterogeneity that has been described in patient tumors based on genetic signatures and clinical behavior.¹¹ These differences between *in vivo* PDX models and *in vitro* systems may help explain the disconnect between results obtained using classical *in vitro* radiosensitivity assays that do not translate into the clinic.

Genetic profiles of PDX are relatively constant during serial mouse passage of these xenografts over long periods (>30 months) using gene expression microarrays and specific gene alterations (e.g. EGFR amplification). However, if PDX tumor cells are placed in serum-based culture for more than five *in vitro* passages, the gene expression profile tends to change dramatically.^{17,33} Short term *in vitro* culture using serum-free, defined NeuroBasal, medium permits easy transfection with reporter constructs (luciferase, GFP) with lentiviruses.³⁴ Subsequent *in vivo* passage has not revealed any morphological, behavioral or gene expression changes. Thus, these tumors are considered to be a more stable platform with respect to serial mouse passage. Nevertheless, very recent single-cell sequencing of 15 breast cancer xenolines demonstrated that engraftment can select for minor clones and that during serial passaging of PDX *in vivo* clonal evolution can occur.³⁵ Similar single-cell sequencing of PDX from other tumor types is needed, but these data in human breast cancer PDX suggest that PDX may not fully recapitulate the genetic heterogeneity of primary human cancers. As such, it is recommended that tissue specimens be frozen down with each passage.

Moreover, PDX tumors may also not fully recapitulate some tumor-host interactions because of human tumor mouse stroma mismatch. While this could potentially occur at interactions mediated by a number of stromal cells (endothelial cells, fibroblasts, etc), the typical PDX system will be unable to model an intact immune system due to the need for an immunocompromised host to allow for xenotransplantation. Thus, the complex interaction of the immune system with a cancer cannot be accurately modeled with the standard PDX model system. With the emergence of several immune checkpoint inhibitors showing clinical promise, including utilization in combination with radiation,³⁶ the inability to test

immunomodulators in PDX models is an important limitation. Recently, however, investigators have reconstituted the human immune system into recipient mice to generate partially and near fully humanized mouse models.^{24,37} Although these mice are now commercially available (e.g. Mixeno™ mice, Crown Biosciences) their exorbitant price tag has prevented the wide scale use of these mice for drug and radiation therapy experiments. Moreover, unless the human donor of the transplanted immune cells is haploidentical with the tumor donor, the combination would be allogeneic in the mouse so that the immune system may attack the foreign tumor, which may make tumor immunotherapy in this scenario irrelevant.²

PDX and Radiation Response Evaluation

Traditional radiobiology assays focus on clonal survival as well as DNA damage (both induction and repair of that damage). However, the predominant model system for this work has been immortalized cell lines with the aforementioned limitations. While determining the surviving fraction 0.2, dose enhancement ratio, and comet assay tail length are important methods of examining radiation response, the direct relevance to the clinic is limited. In many cases, immortalized cell line experiments using these traditional radiobiological methods are followed by *in vivo* studies using the same cell lines implanted in athymic nude mice, which do not necessarily correlate with the *in vitro* data.³⁸ Yet, as discussed previously, the clinical applicability of immortalized cell lines, even when implanted in mice, is questionable. As such, there has been a steady increase in the use of more biologically relevant PDX model systems for radiation research.

The Mayo Clinic (Rochester, MN) has embraced the development and widespread use of PDX in cancer research. The Mayo Clinic's high grade glioma PDX program, first developed by Dr. C. D. James and carried on by Dr. J. Sarkaria, has been used to examine radiation responsiveness in orthotopically (intracranially) implanted GBM PDX.^{39,40} Radiation response was determined by treating tumors using whole brain irradiation of the mice with various treatment regimens (2 Gy twice daily for 5 days, 2 Gy three times per week for 2 weeks, or 2 Gy three times per day for 2 days). Survival studies revealed that the GBM PDX could be inherently radiation resistant or radiation sensitive, regardless of epidermal growth factor receptor (EGFR) amplification status or whether the O⁶-methylguanine-DNA methyltransferase (MGMT) promoter was methylated.^{39,40} As such, simplistic biomarkers (i.e., expression or modification of one protein or gene) were unable to reliably predict radiation sensitivity for GBM-PDX. Instead, molecular profiling coupled with the phenotypic information generated by direct radiosensitivity testing of the PDX is likely to produce more meaningful results and generate appropriate targets for drug development (**Fig 2**). Conformal/stereotactic mouse radiation delivery systems are available that are more clinically relevant as well.^{36,41} Nevertheless, *in vivo* PDX testing is likely to be a later preclinical assay in a research program developing new therapies because PDX high throughput testing is cost and time prohibitive.

3D Culture Approaches for PDX and Radiosensitivity Testing

Due to the cost and infrastructure requirements for PDX testing in mice, there has been particular interest in generating cell-based culture systems that can harness the patient

tumor-like characteristics of the PDX while providing the ease of more traditional cell culture, with fewer of the aforementioned limitations. Many believe that cancer stem cells or at least cancer stem cell-like cells (CSC's) can be propagated from PDX when grown in appropriate conditions.^{2,25} In the example of GBM, CSC's can be grown as neurospheres using NeuroBasal defined media that does not contain serum.^{33,42} When grown in low oxygen tension with low glucose, the cell culture conditions will more closely resemble that encountered by an intracranial tumor. However, the ability to quantify radiation cytotoxicity in such a model system can be challenging. One approach has been to treat growing neurospheres with radiation and, following an appropriate incubation time, the neurospheres are forced to attach by the introduction of serum to the media. "Attached" neurospheres can then be fixed and stained for quantification (**Fig 2**).⁴² Others have simply tried to quantify tumor-sphere diameter following radiation, which was recently shown for PDX cells of small cell carcinoma of the cervix.⁴³ These authors showed consistency in radiation response between the tumorspheres and their paired *in vivo* PDX tumors. Moreover, they discovered HIF-1 α upregulation in the radiation resistant PDX. Alternatively, direct measurement of tumor-sphere growth potential can be performed following radiation using an *in vitro* limiting dilution tumor-sphere formation assay.⁴² Another group has used *ex vivo* radiation treatment of PDX cells to assay Rad51 foci as a predictor of homologous recombination DNA repair deficiency. They found that this *ex vivo* assay could predict *in vivo* poly (ADP-ribose) polymerase (PARP) inhibitor efficacy in ovarian cancer PDX.⁴⁴

An attractive approach could be the culturing of PDX cells in three-dimensional (3D) model systems. Over the past several years, the lab of Dr. Nils Cordes has demonstrated that the 3D culture conditions produce data on radiation sensitivity that is distinct from that of monolayer culture.⁴⁵⁻⁴⁹ Although his team's studies have used immortalized cell lines rather than PDX, 3D culture of PDX cells may more closely resemble physiological conditions. Over a decade ago, Fiebig et al. described a multilayer clonogenic assay approach using CSC's and very early passage PDX cells (typically first or second passage).⁵⁰ The focus of the manuscript was predominantly that of chemotherapy response in clonogenic assays vs. *in vivo* assessment vs. clinical response. The *in vivo* studies had the highest predictability for patient response, though the clonogenic assay was still useful for predicting chemotherapy response. Importantly, they did perform some radiation testing, specifically in colon carcinoma and lung carcinoma showing considerable radiation resistance in the colon PDX cells and relative radiation sensitivity in the lung PDX cells.

In recent years, there has been increasing commercial interest in developing novel 3D systems³ to facilitate high throughput testing of drugs, e.g. microcarrier beads, hollow-fiber bioreactors, and cellular spheroids. Tumor spheroid models involve cell aggregation by using spinner flask, rotary chamber, pHema-coated plate, and hanging-drop culture techniques. Most 3D culture systems utilize either synthetic (Alginate, Hydrogel) or animal-derived matrix (Matrigel™, collagen) as scaffolds. Although useful, these systems pose many limitations, particularly lack of tumor cell heterogeneity as not all cells form stable spheroids. In addition, tumor spheroid models are difficult to adapt for automated imaging and high-throughput screen (HTS) analysis due to poor reproducibility. To try to overcome this, we have begun working with the HuBiogel™ 3D MicroTumor culture system (Vivo

Biosciences, Inc., Birmingham, AL) (**Fig 2**) consisting of a natural extracellular matrix (ECM) containing collagen-I, laminin, collagen-IV, collagen-III, entactin, and heparan sulfate proteoglycans and lacking all major known growth factors (GFs).⁵¹ Since HuBiogel™ is neither angiogenic nor mitogenic, the growth of PDX cells, including multiple cell type growth, is less subject to the selection pressure of other ECM preparations, such as Matrigel™, that contain intrinsic growth factors and lack key stromal collagens.

Avatar and Proband Modeling

While discovery of mechanisms of radiation resistance and sensitivity is crucial, as is the identification of novel radiation modifiers, it is abundantly clear that not all tumors are created equal. In fact, we generally do not lack for potential therapeutic agents, but what we lack is appropriate patient selection for those agents, be it stand-alone therapy or in combination with radiation. The ultimate goal for many investigators utilizing a PDX system is to generate a testable system that can be used to inform clinical decision-making.²⁸ The so-called “tumor avatar” represents the situation in which a patient has a PDX generated from their tumor that can be used as a therapeutic guide for response assessment (**Fig 3**).^{19,20,28,52} A pilot study published in 2011 described the use of pancreatic cancer PDX for treatment guidance in patients, which demonstrated feasibility and success in advanced cancer patients.⁵³ A recent study by Crystal et al. showed the utility of PDX testing for identifying appropriate drug combinations in lung cancer patients that could not be deciphered from genetic testing alone. Indeed, the authors stressed the importance of direct drug testing of PDX.⁵⁴ In fact, numerous companies have been developing such systems. While tumor avatar creation and testing may conceivably work for individual patients who have relatively slow growing tumors (that temporally allow for parallel propagation of tumors in mice), a true avatar is not possible for very aggressive tumors, such as GBM, where initial passage of the PDX may require up to 6 months. With a median survival of 14 months for GBM patients, it is unrealistic to expect that a tumor avatar can be produced and tested in time to inform a clinician. Thus, the proband model concept has been suggested as a more practical solution to this problem (**Fig. 3**). With a proband approach, the PDX developed from a patient does not directly inform the clinician regarding that specific patient's therapy. Instead, a patient's tumor is “matched” to an existing, well-characterized (both molecularly and phenotypically) PDX. This model assumes that the PDX “library” adequately represents the spectrum of human disease and that an individual's PDX can be matched to human tumors by some molecular means, such as genomic or transcriptomic profiling.

Incorporating PDX into drug discovery and translational research programs is garnering support. In fact, the National Cancer Institute has developed a PDX screening program for preclinical drug development in pediatric cancers. This Pediatric Preclinical Testing Program suggests a promising translational approach is possible and is particularly useful for rare tumors, such as pediatric cancers.^{55,56}

Kinomics and Other “Omic” Testing

Critical to the establishment of a proband model system is the collection of molecular profiling and reliable classification of PDX tumors as well as human tumors for potential matching.²⁰ There have been many reports detailing the molecular characteristics of PDX systems for a variety of tumors.^{17,57-60} By comparing these tumors to the patient “omic” characteristics within the TCGA, one can determine how well a particular PDX cohort represents part of the spectrum of clinical disease. More importantly, the utility of a proband model system for clinical decision-making requires the capacity to link a patient's tumor to the appropriately representative PDX.

Beyond the use of genomic and transcriptomic approaches for proband assignment, molecular characterization coupled with phenotypic knowledge gleaned from direct testing of therapeutics in the PDX is an excellent hypothesis generating strategy. Indeed, we have used global kinase activity (kinomic) assessment of GBM PDX in the setting of known PDX sensitivity or resistance to temozolomide as well as radiation.⁶¹ This kinomic profiling involved the direct measurement of kinase activity in GBM-PDX tumors using a multiplex *in vitro* kinase assay microarray platform (PamStation®12, PamGene International, The Netherlands) in the UAB Kinome Core. Radiation resistant GBM-PDX displayed distinct kinomic profiles, which identified potential kinase targets for drug development as radiation modifiers.⁶¹

The molecular characterization of PDX has also found utility in biomarker development in the setting of the parallel clinical trial.^{20,62} Parallel clinical trials, also known as “co-clinical trials” or “human surrogate trials,” refer to the use of a trial in mice with tumors, such as PDX, which is coupled with an actual human clinical trial. Due to the limitations in obtaining sufficient clinical specimens and lack of statistical power, particularly in early phase clinical trials, the use of a parallel phase II-like PDX trial allows the completion of molecular analysis with adequate sample number to support the development of a clinical biomarker. As such, PDX “clinical trial hospitals” are being developed specifically for this purpose (e.g., Crown Biosciences and Shanghai Institute of Materia Medica, Chinese Academy of Sciences Mouse Clinical Trial Center or SCMC). Time will tell how successful this type of endeavor will be.

Conclusions

PDX represent the most advanced and clinically representative human tumor culture system available. Although radiation studies using PDX have been limited in scope and number as compared to chemotherapeutic studies, the future of molecularly-targeted agents and radiation therapy seems to indicate that PDX approaches will play a role in preclinical model systems.⁶³ With the more widespread availability of image-guided and conformal radiation delivery systems for mice (e.g. small animal radiation research platform [SARRP], Xstrahl and X-RAD 225Cx, Precision X-ray), investigators are now able to perform focused irradiation studies on orthotopically implanted tumors.^{36,41} While *in vivo* studies using PDX are not compatible with high throughput testing, the use of 3D cultured PDX and human CSC's may bridge the gap between monolayer studies and animal testing.

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Figure 1. Patient-Derived Xenograft Advantages and Challenges. Schematic of derivation of Patient-derived xenografts (PDX) is shown. Passage (P) number is indicated. The number of passages that can be utilized depends on the tumor's genetic stability as well as the host type. Athymic nude mice tend to maintain the PDX genetic stability longer than more severely immunocompromised mice though at the expense of lower take rates. At each passage, PDX tumor is typically frozen down. Advantages of PDX over immortalized cell lines are shown. Challenges that are encountered with a PDX program are listed.

Figure 2.

Approaches to Radiation Biology Study using Patient-Derived Xenografts. Patient-derived xenograft (PDX) tumors and cells can potentially be used for radiation studies. PDX tumors can be directly tested *in vivo* for tumor growth and animal survival using mouse irradiators. With the advent of conformal irradiators such as the small animal radiation research platform (SARRP, Xstrahl and XRAD 225Cx, Precision X-ray), orthotopically implanted tumors can be specifically treated with image guidance and/or monitoring. PDX tumors are expected to contain cancer stem cells or stem cell-like cells (CSC's) that can be maintained as tumor spheres when cultured in non-differentiating media. An example of Glioblastoma multiforme (GBM) PDX neurospheres is shown. PDX tumor cells can also be implanted into extracellular matrix material as 3D culture. As with tumor spheres, these 3D cultures should be maintained in non-differentiating media (i.e., lacking serum). An example of GBM PDX cells embedded in HuBiogel 3D MicroTumor beads (Vivo Biosciences) is shown. Molecular profiling using genomic, transcriptomic, kinomic, *ex vivo* DNA damage assays can generate hypotheses for radiation sensitivity and resistance that can then be directly tested using various molecular and therapeutic means (lentiviral infection, drug therapy, etc.).

TUMOR AVATAR APPROACH

PROBAND APPROACH

Figure 3. Clinical-Translational Model Incorporating Patient-Derived Xenografts. Two patient-derived xenograft (PDX) clinical-translational models are shown. The tumor avatar approach (Top panel) is one in which the patient's tumor is used to generate a PDX that will directly inform his or her own therapy. A schematic is shown for one potential tumor avatar design in which an individual patient and a PDX derived from this patient's own cancer are given standard of care (SOC) therapy, such as chemotherapy (chemo) and radiation (XRT). When the PDX develops resistance to the SOC, the resistant PDX is then expanded and

tested against potential second line therapies (Tx1-4). The winning therapy (indicated by trophy) is then selected for second line therapy for the patient when they develop tumor recurrence following SOC. A tumor proband approach is shown schematically (bottom panel). In a proband model system, a cohort of PDX are utilized that represent the spectrum of human disease for that tumor type. These PDX are molecularly profiled and tested against various therapeutic regimens, such as irradiation (XRT), chemotherapeutics, and small molecule inhibitors. Ideally, molecular profiling is performed longitudinally (i.e., pre- and post- therapy) as molecular profiles likely change due to tumor adaptation/response. Well-characterized PDX will generate a library of profiling and phenotype information that can be used to inform clinical decisions as follows: a patient's tumor is molecularly profiled and matched to a particular or multiple PDX and known therapeutic sensitivity information is then passed back to the clinician for selection of proband-directed therapy.

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