



Patient-Derived Xenografts use in Cancer: A Review

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Abstract

Cell line based disease modeling, with its homogeneity and transformations caused by years of growth in petri dishes, has been underwhelming with limited information found using them successfully translating to patient care. Because of these significant problems presented by historical modelling of cancer there has been increase interest in patient-derived xenographs (PDX), which is the implantation of a portion of patient tumor into an immunodeficient mouse, as an improved model for cancer. Improved modeling attempts to increase bench to bedside transition, to improve patient care. PDXs maintain significant tumor heterogeneity and microenvironment similar to donor disease. This article reviews the use of this model to assist with direct patient care, biomarker studies, drug studies, and advancement of other techniques. As well, this article reviews the avatar/super avatar model, proband model, co-clinical trials, preclinical testing, as well as immunomodulatory considerations. This review of the model includes are historical considerations as well as current studies in the field including advantages, correlation ability, an example of subcutaneous establishment method, factors effecting establishment, characteristics, applications, limitations, shortcomings and future direction table 1.

Advantages of PDX

In the late 1960s, the NCI established the NCI-60 Human Tumor Cell Lines Screen in an attempt to create a panel of cell lines to aid in preclinical testing for new drugs. By 1969, the first patient derived xenograph (PDX) was established, using athymic ‘nude’ mice. The first PDX was established by Rygaard and Povlsen [1] when they implanted patient colon cancer cells subcutaneously, thereby establishing the potential that human tumors could be grown in immunocompromised mice. At the time, PDXs were not deemed as superior to cell lines because they were more challenging to grow and it was not known if such models conferred any predictive advantages compared to cell lines. Since that time, human cancer modeling has changed significantly in the attempt to better reflect human disease. Cell lines have been found to be less predictive than anticipated.

Since the initial description in 1969, interest in using PDXs as a pre-clinical cancer model has been inconsistently maintained. For most of that time, interest has focused mostly on cell lines. Unfortunately, cell lines are associated with significant caveats; in some cases they have been maintained in culture for decades and no longer resemble the tumor tissue of origin from which they are derived [2]. Cell line growth can be strongly influenced by the cell culture conditions in which they are propagated, which may have very little in common with the actual tumor environment of origin. Research has shown that tumors have both intratumoral heterogeneity [3] and presumed subtype heterogeneity [4], as differences in between tumors as well as between cells within the same tumor, which may not be reflected in homogenous population that can characterize cell lines after multiple passages.

PDXs have been shown to form tumors containing heterogeneous populations and spatially distinct clones [3]. Cancer drug advancement from the preclinical stage, based on cell line screening, to successful patient treatment applications has been problematic, and only approximately 7% of drugs under evaluation successfully advance past phase II clinical trials [5]. As an example, cell lines predicted arsenic trioxide as a treatment for small cell lung cancer (SCLC). However in PDX models it not as effective as in the cell lines and ultimately was not effective in patients. This suggests that PDX models are superior to cell lines for screening this anti-cancer agent [6].

Xenografts derived from cell lines have many of the same problems, specifically, mutation and homogeneity, as cell lines. The hypothetical advantage is that by being placed in mice, the tumor cells and the drugs being tested are interacting in a more complicated and “real world” environment then if they were being testing in a Petri dish.

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Table 1: Factors effecting establishment rate.

Increase establishment	Decrease establishment
Chemo resistance [13]	Chemo responsiveness [13]
Tumor size over 3.5 [14]	Operative type [14]
Aggressive histologic grade [15]	Operative time [14]
Lymph node involvement [15]	Oncogene expression [14]
>5 CTCs/7.5 mL	T cell infiltration [15]
Decreased RFS/OS	Cell suspension [16]

Xenografts are an undoubtable superior model to cell lines: they help determine response to therapies in a compartment of a living animal, researchers hope in xenografts to co-opt the mouse microenvironment to better answer questions about human disease. PDXs have these strengths plus the human tumor microenvironment, with its well preserved blood vessel vascularization, pericyte coverage, tumor-associated macrophages, cancer-associated fibroblasts, and extracellular matrix components [7] that lend a more realistic environment to the model.

An alternative strategy to model cancer is using genetically engineered mouse models (GEMM). The first GEMM was OncoMouse, a mouse with a MTV/myc fusion gene, that was created in 1984 [8]. One advantage of these models is that the mice are immuno competent, with spontaneously or virally created tumors that are used to model disease. One issue with these mice is that the tumors are always related to the fusion gene or specific mutants and do not provide a full range of disease that is typically present in patients. These models are limited to only a small amount of cancers as they require a promoter gene or fusion proteins. GEMMs also have the problem of multiple synchronistic lesions that can confuse the end result. GEMMs created cancers can become addicted to the oncogene that created them, enabling them to act different than naturally occurring cancers [9].

Historical Considerations

PDXs have been around since 1969. However at the time cell lines were thought to be easier to use and at least as predictable. Therefore, PDXs as models for human cancer research were not widely used. However, because of the low fidelity of cell lines, researchers have started to look back into other forms of modeling. In 2006, Hidalgo established subcutaneous PDXs in a study of pancreatic cancer in nude mice [10]. They essentially rejuvenated PDX modeling. It was hoped that newly established PDXs are able to address some of the problems with long established cell lines. Subcutaneous PDXs are easy to establish as they require little expertise, are easily accessible, and easy to measure their progress, but there are concerns about the lack of appropriate microenvironment. Interest has expanded to orthotopic models hoping they better represent systemic disease, as subcutaneous PDXs grow locally but do little else. Orthotopic models have been around since 1984 when Wang, et al. [11] injected colon cancer cell suspensions into the descending colon of nude mice. It was not until 1991 that intact tumors were placed orthotopically for the first time. Fu et al. [12] placed patient derived tumors from human colon cancer back into the colons of mice and the tumors reliably showed increased rate of metastasis, in disease specific site.

Increased interest in PDX models can also be attributed to increased engraftment rates. As mice get increasingly immunocompromised, the engraftment rate has gone up. The first immunocompromised mouse was the nude mouse; these were first developed in 1962.

These mice lacked a thymus and subsequently T cells. The first PDX experiments were performed in these mice, and they continue to be clinically useful as their intact B-cells allows for part of their immune systems to behave normally. Severe Combined Immunodeficiency (SCID) mice were introduced in 1983 and lacked mature T and B cells. Non-obese diabetic SCID (NOD-SCID) mice lack mature T and B cells while also having lower levels of NK cells and a defective complement system. At this time, the most immunocompromised mouse model is NSG, NOD/SCID/IL2Rγ^{null}, or NOG, which has led to an even greater increase in engraftment rates in difficult to grow tumors. They are thought to have close to 100% engraftment rate in hematologic malignancies.

PDX Establishment

Once the tumor is removed from the patient, after appropriate clinical tissue is taken, the remainder of the tissue is transferred on ice to where the PDX is to be established. The tumor is cut into 5mm by 5mm chunks to be placed in the mice. Once the mice are anaesthetized, and shaved, for subQ placement, using forceps to lift up the skin to ensure no peritoneal violation a small 1cm incision is made in the skin with scissors. The subQ is probed to create a pocket, the tissue is placed inside the pocket and the skin is closed with adhesive, suture or clips. Then the anesthesia wears off and the mouse continues its normal activities. Different factors effect PDX establishment in different ways.

Characterization of PDX model

PDX have been shown to be very good representations of their parent tumors. Genetically they have significant clonal complexity [17]. Owonikoko TK, et al. [18] published that a small cell lung cancer PDX that kept short tandem repeats (STR) through 2 PDX passages. Cheung PF, et al. [6] published an hepatocellular carcinoma (HCC) PDX that had no significant changes after serial propagation. PDXs have shown 92-97% identical genetic aberrations when patients tumors are compared to passage 1, with 92-94% showing the same somatic aberrations with 3-10 somatic mutations [19]. Genetic testing of 56 HCC PDXs has shown on average 8033 protein altering changes, with .05% being stop change in frame deletions, .8% being stop change single-nucleotide polymorphism, 1% being frame shifts and the rest being non synonymous [20] relatively few considering cancers are constantly transforming. In one study, the PDX-to-parent gene amplification concordance was high at 94-100% in FGFR1, MET, and ERBB2 after 12 passages. Protein expression showed moderate concordance at 78%, for PTEN and MET, while showing just average concordance in ERBB1 and ERBB3 at 59-75%. However, ERBB1 and ERBB3-positive tumors showed 81-87% concordance with PDX compared to ERBB1 And ERBB3-negative tumors showing 31-40% [21] concordance, showing a progression of disease as opposed to regression. PDX modeling showed that the aberrations lost during passages were most likely due to lack of selection by host and any gains seemed to be of tumor progression rather than model-induced factors [22]. PDXs have been shown to form tumors with heterogeneous populations and spatially different clones[3]. Subcutaneous placement has been shown to grow well and keep their genetic makeup, but are often found to be encapsulated and rarely metastasize [12]. In tumors with TP53 abnormalities, PDX models genetic makeup was more like the metastasis than to primary renal cell carcinoma (RCC) when compared between the two [23].

Tumor microenvironment was well preserved with blood vessel vascularization, pericyte coverage, tumor-associated macrophages,

cancer-associated fibroblasts, and extracellular matrix components [7]. Lymphoid cells and lymph vessels were seen in PDX models using nude mice but not NOD SCID gamma (NSG) mice. Thus suggesting that variations in the immune systems in these models may have additional effects on tumor than just lack of ability to use immunotherapy. Patient-derived endothelial cells can form tumor vasculature in early passages [7], showing extension from the PDX to the host. Unfortunately murine cells have been seen to associate with the stroma after the first passage of NOD-SCID mice [24], showing early passage PDXs are required to maintain the concordance with patient disease.

Avatars

The patient avatar method is when a PDX model is established to test a wide variety of drugs and then use that information in the same patient the PDX was established from. These avatars are being used to specify second-line therapy, therapy after all other care has been exhausted, or if a therapy does not exist.[25] Hidalgo et al. [26] showed 88% of patients had expected results (based on PDX predicted results) as compared to 10% that had unexpected results in a second-line treatment setting. Stebbing J, et al. [27] published PDX avatars in sarcomas, that showed there was a 76% growth rate and 81% concordance rate between patients and their PDXs. They also described a 27% death rate during the time taken for PDX establishment. In total Stebbing describes a 44.8% response rate in an intent-to-treat analysis. Patient avatars have been used with good success in rare diseases that have variable response rates and few treatments. PDXs have been used successfully in a patient with rapidly growing adenoid cystic carcinoma disease becoming stable. During the 6 months of treatment with IGF1R inhibitor, the peritoneal disease stopped growing, until the study was stopped secondary to brain disease. IGF1R would not have even been considered for use before the PDX results [28]. In a patient with SCLC, the avatar models have been shown to reveal actionable responses whereas gene sequencing did not [29].

Limitations

Although many uses for PDX have been demonstrated, it is not without limitations. If PDX establishment is successful, which has been shown to not always be the case, it normally takes anywhere between 6 months and 1 year to generate the avatars [26]. Testing new drugs on previously established frozen tissue takes approximately an additional month [unpublished data] on top of the 1-2 months for the PDXs to be of appropriate size and measure response. The development of PDXs is time intensive as well as expensive with costs ranging from mice acquisition, continued care, and for anesthesia. The costs can add up quickly; our lab spends approximately \$100USD to acquire a single mouse, then \$1USD a day, and there is a variable establishment rate and growth rate.

Increasingly immunocompromised mice appear to have increased establishment rates. Immunotherapy however is difficult to study in immunocompromised mice. While PDX engraftment can bring a small amount of human stroma tissue, the decision of where to place tissues have been variable but there are groups that believe that orthotopic placement gives a better microenvironment to help replicate the original tumor biology [30]. Murine stroma has key differences compared to human stroma in the ligand repertoire that may be critical in mimicking the true microenvironment [31].

Zhang, L., et al. [32] described a significantly increased rate of

unexpected lymphomas. They described a 32% lymphoma rate in NOD/SCID mice with 88% being of human origin. The increased rate of lymphomas was tumor specific they describe a 19% rate of lymphoma in the gastric cancer PDX, while only in 2.3% in colorectal cancer.

Super Avatars

It has been postulated that a 'super' avatar would help solve some of the problems avatars offer. 'Super' avatars are created using hematopoietic stem cell transplantation as well as orthotopic tumor, basically the patient's immune system as well as the tumor is transplanted into the mice. The hope of this is that the transplantation of a human immune system will allow for immunotherapy to be tested [33]. Hematopoietic stem cell transplantation has been used with cell lines to try to identify antibody treatment for cancers that do not have them [34]. To the best of our knowledge super Avatar's have not been established but their theoretical promise is interesting. As being able to further investigate immunotherapy with PDXs would increase our repertoire as well as allow fully humanized therapy in mice. This would allow quick delivery of drug from *in-vivo* testing to phase 1.

Again, while the hope of super avatars is interesting they have yet to be established and show efficacy. The addition of being able to test the immune system is exciting, as regular avatars completely neglect this aspect of care. Super avatars will require stem cell transplant, which will be costly, and require advanced expertise in specialized centers. It is not known if the co-transplantation will affect the PDX establishment rate, as humanized immune systems may reject the tumor.

Correlation

While it would be great if we were given infinite time and attempts to cure patients, it just isn't realistic. When considering a specific *in-vivo* model, how well it represents patient disease and can answer relevant clinical and scientific questions. PDXs while they do have their limitations are a very good representation of patient disease. Along with genetic similarities described earlier, they behave in similar ways. For instance, PDXs have shown similar response rates in colorectal cancer; a 39% response in PDXs to irinotecan [35] compared to 19-32% previously described [36] in patients. As well, cetuximab has shown 29% response rates [35] in PDX models compared with 11% response in patients [37]. Furthermore, in non-small cell lung cancer (NSCLC) paclitaxel has been shown to have a response rate of 16% in PDXs [38] compared with 21-24% in patients [39] and cisplatin plus vinorelbine has shown 28% response rate in PDX compared to 24% response rates in patients. Most investigators are limiting passages to around three in an attempt to limit genetic drift from parent tumors, as well as murine overgrowth. When there are discrepancies between PDXs and patients, the PDX tend to drift towards more aggressive phenotypes.

PDXs have been used in a large range of modeling of both common and rare diseases. Most of the morbidity and mortality of cancer comes from metastases. The ability to better understand metastases would greatly increase our ability to treat them. The metastatic ability of PDXs is greatly improved by orthotopic placement. Orthotopic tumor models in neuroblastoma tumors were shown to have similar metastatic potential as the patient's tumor, progressing to but not beyond stage 3 [7]. Orthotopic PDX models using tissue slices have shown to have metastasis in the same locations as the primary patient

in RCC including the bone which had not been seen previously [40]. In an orthotopic model of breast cancers there was a variable metastasis rate between 38% and 100% depending on the tumor line [41]. We presume the orthotopic PDX model is a better model of a patient's disease progression, which should be an improved representation of therapeutic result. Therapeutic differences between orthotopic and subcutaneous models needs to be established as they have never been directly compared.

PDX Utility in Immunomodulation Trials

PDXs have the potential to be used for preclinical trials involving immunomodulation, such as anti-IL-6 antibody therapy. In PDX models of head and neck squamous cell carcinoma (HNSCC), anti-IL-6 antibody treatment decreased tumor growth. Interestingly, this effect was seen in PDX model from untreated HNSCC but was not seen in PDX models representing resistant disease. Anti-IL-6 antibody treatment also decreased the fraction of cancer stem cells and reduced the recurrence in this PDX model of HNSCC [42]. Although the potential for PDX models to predict sensitivity and resistance to therapeutics requires further validation, this is promising for future preclinical trials, as it was not assumed that the immunodeficient mouse was a good medium to test immunotherapy.

One potential drawback of immunomodulation in PDX models is the use of immunodeficient mice. However, reports have shown that using different mice might allow for studying immunomodulation in PDX mice. Implanting non-disrupted pieces of human lung tumor into NOD-scid IL2Rgamma (null) mice showed maintenance of the tumor microenvironment, including effector memory T cells. These tumor-associated T cells migrated out of the tumor and could be recovered from mouse spleen, lung, and liver [43]. Another potential strategy to combat the lack of immune system in PDX models is to humanize the PDX model with human immune system using human bone marrow, liver, and thymus [44].

Another anticancer strategy that can be studied with PDX models is the use of oncolytic viral vectors to express anticancer therapeutics. Treating colon cancer PDX nude mice models with adenoviruses carrying tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) demonstrated inhibitory effects on tumor growth without evident treatment-related toxicity [45]. Similar results were found in PDX in intracranial tumors [46]. Adenovector expressing small hairpin RNA (shRNA) targeting Bcl-XL in rectal cancer PDX model suppressed tumor growth [47]. Using cancer-selective replication of adenovirus, enforced expression of interfering long non-coding RNA (lncRNAi) decreased tumor growth in PDX models of hepatocellular carcinoma in nude mice [48]. As these reports have shown, PDXs are promising for use in pre-clinical studies involving many different genetic strategies based upon adenovector targeting.

Biomarkers

Biomarkers are also a significant area of study that PDXs have been able to assist with, PDXs have showed the ability to have appropriate biomarkers just as human cancers. 81% of HCC PDXs showed elevated alpha fetoprotein (AFP), which is used to evaluate tumor burden in patients [20]. Unfortunately, molecularly targeted therapy has only shown a response between 10-20% in unscreened patients [49]. As a significant amount of targeted therapy has not had the expected results in human tumors, this leaves a large area of research needed to establish biomarkers that will better predict clinical response. PDX modeling has been used to test biomarkers and

responses for preclinical trials, after a failed phase 1 trial secondary to lack of biomarker in targeting therapy in HER-3 gastric cancer. PDX modeling showed that anti-HER2 and HER3 monoclonal antibodies work synergistically *in vivo* similarly to *in vitro* and show similar downstream molecular effects [50]. This may allow these biomarkers to be used in a second phase 1 trial, for more appropriate patient selection. PDXs have been used to determine response to drugs based on chromatin and determining which chromatin regulatory genes will respond to known therapies [51].

PDX models have been used to look for biomarkers suggestive of radiation response [52]. PDX models were used to evaluate response to TKIs based on EGF mutations and MET expression markers [53]. PDXs have also been established in rare and high-risk cancers such as cholangiocarcinoma in order to test which TKI is most potent [54]. PDXs have shown similar tumor markers as host tissue hopefully allowing biomarker investigation in the future.

Preclinical Testing

Drug testing

An improvement from the 7% success rate in clinical trials [55] in cancer from phase I to approval is paramount. The impact of PDX models on this number is not known. PDXs have also been used to examine the effects of hypoxia-targeting drugs and shown them to be effective. In untreated PDXs, hypoxia percentage remained stable in both subcutaneous and orthotopic PDX models through serial generations [56]. Thus showing the treatment is possibly effective and that the model is consistent when repeated. High-risk prostate cancer has traditionally been a hard disease to model as prostate cancer is very heterogeneous, and high-risk disease which causes most of the morbidity, is rare. PDXs have been able to be established using thin slices with adjacent tissue being pathologically confirmed as aggressive [57]. With increased interest in drug testing other models have arisen, one of which is live tissue sensitivity assay (LTSA), which has attempted to show drug sensitivity using thinly cut slices from PDXs. If this is validated as a faithful representation of human tissue biology, it would help diminish lead time testing [58]. Along with this early passage cell lines have been reinvestigated, as cell lines have been difficult to establish in the past, PDX models have been used to establish new cell lines with a reported success rate of 100% compared to the success rate of 10% when cell lines are passaged directly from tumors. Cell lines established from PDXs remained human with significant heterogeneity and variable growth patterns that were similar between cell lines and the PDXs [59].

Co-clinical

One of the hopes of using PDX modeling is that they can be used in a proband model [60], which helps identify markers to predict which PDX model the tumor will behave like and choose the treatment that worked in the similar PDX. While avatars might help patients in the immediate time period while also helping to identify markers that may help skip the need for PDXs in the future, the proband model would hopefully be able to model most all subsets of cancer out there to better predict results for individual patients. While preclinical testing and the proband model will likely advance the treatment of more common diseases in the future, PDXs have been a great resource at this time for rare diseases as they have the potential to help to identify treatments that would have otherwise taken significant amounts of time to find and test.

Phase 1

Phase 1 trials are used in human subjects to study how much of

the drug people can get safely. PDX models have been used in SCLC to test drug toxicity levels of different checkpoint inhibitors; they did this by measuring levels of drug in the plasma as well as in the tumor. They were able to see significant reduction with new agents that acted on similar targets, from this they inferred that the toxicity would be lower, as less of the drug was required and identified potency differences [61]. However at this time other than measuring mouse well-being with weight and overall appearance it is difficult to study other side effects.

Future Directions

We believe that PDXs are a powerful model, which for good reason have increased in popularity by allowing a better representation of patients' original tumor. The increased use of PDXs will likely lead to significant advances towards the overall goal of improving cancer treatment. PDX models have been used to look into other ways to advance science.

Summary

PDX is a powerful tool used in modeling patient tumors. In its early adoption, it has already enabled scientific advances and has been set up to greatly improve them with time. As it is still evolving, there are some aspects that need to be further investigated. The availability of representing patients accurately with no consequence to them is imperative. It has been used in many different settings, and its potential to help increase drug development and knowledge of disease is invaluable. While it is not without limitations, as more and more PDXs get established, PDXs are likely to increasingly augment cell culture as a medium as they are more representative of tumor biology in patients. PDXs have opened researchers' perspectives to re-examine their methods in order to increase the fund of knowledge, making this an exciting time for science and the future achievements that will come out of it.

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