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Prioritizing therapeutic targets using patient-derived xenograft models

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Abstract

Effective systemic treatment of cancer relies on the delivery of agents with optimal therapeutic potential. The molecular age of medicine has provided genomic tools that can identify a large number of potential therapeutic targets in individual patients, heralding the promise of personalized treatment. However, determining which potential targets actually drive tumor growth and should be prioritized for therapy is challenging. Indeed, reliable molecular matches of target and therapeutic agent have been stringently validated in the clinic for only a small number of targets. Patient-derived xenografts (PDX) are tumor models developed in immunocompromised mice using tumor procured directly from the patient. As patient surrogates, PDX models represent a powerful tool for addressing individualized therapy. Challenges include humanizing the immune system of PDX models and ensuring high quality molecular annotation, in order to maximise insights for the clinic. Importantly, PDX can be sampled repeatedly and in parallel, to reveal clonal evolution, which may predict mechanisms of drug resistance and inform therapeutic strategy design.

Keywords

personalized medicine; patient-derived xenografts; genomics; targeted therapy; therapeutic targets

1. Introduction: Identification of therapeutic targets in the clinical setting

Through our improved understanding of cancer biology, identification of molecular drivers of cancer growth, and the development of targeted therapeutics, we have an increased ability

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to deliver treatment matched to a patient's cancer. The reality, however, is that for the majority of patients, this approach is still beyond their reach. The process of personalized medicine focuses on treating a patient as an individual, rather than as a representative member of a group of patients with similar histological designation, as has been the historical mechanism for assigning treatment[1]. However, this approach entails significant challenges in terms of logistics and identification of the best model systems in which to validate the utility of personalized therapies. The use of patient-derived xenografts (PDX), or human tissue transplanted into immune-deficient mice without any intervening *in vitro* culture step, provides powerful models in which to determine the efficacy of therapies targeted to specific molecular aberrations[2].

In the past five decades of cancer therapeutic discoveries, the way in which a cancer case has been described and matched to treatment has focused on the organ in which the cancer was thought to have arisen[1], the histopathologic appearance of the cancer tissue and draining lymph nodes and the staining of between one and ten protein markers present on or in the cancer cell. Indeed, apart from a number of molecular tests involving the analysis of one or two genes, such as the routine use of *in situ* hybridization analysis to determine amplification of the *HER2* gene in breast cancer or DNA sequencing to determine mutations in *KRAS* in lung cancer or colorectal cancer[4]; or in melanoma or colorectal cancer, histopathology and immunohistochemstry underpins the majority of treatment decisions for many for many patients today.

We are currently in the middle of the most extraordinary technological revolution[6], which has led us from the mammoth task of proposing to sequence the first human genome, predicted to take 15 years and cost three billion USD, to the current availability of whole genome sequencing (WGS), of an entire genome (or a cancer genome) in only a few days, for the cost of around one thousand USD. Indeed, genomic technologies, such as high-throughput sequencing of DNA, RNA (RNASeq), microRNA and the epigenome, now provide the first systematic approaches to discover the genes and cellular pathways underlying disease[6]. Although these technologies provide a tremendous opportunity, being able to read individual base pairs and compare them with a reference sequence does not tell us what we urgently need to know: who will get cancer, what type and when and how should that cancer best be treated? There is hope, however, that companion technologies that allow us to determine gene expression and epigenetic marks, or silencing or accessibility of the genome, will enhance our ability to interpret gene sequence variations.

Thanks to exponential improvements in the speed and depth of DNA sequencing, next-generation sequencing (NGS) can analyse entire human genomes in days, at a reassuring read depth[7]. Sequencing a cancer genome is more complex than a germline genome, due to the variety of complex aberrations found in cancer, including multiple gene copies, structural changes, epigenomic changes and intra-tumoral genetic heterogeneity[7]. This complexity necessitates greater read depth, or coverage (how many times a specific region has been sequenced by unique reads with a different start/end site/read length), with a median coverage of 50× (excluding duplicate reads), rather than the 30× generally accepted for standard germline genomes. Criteria for ensuring quality NGS data and interpretations

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are being addressed by the Next-generation Sequencing Standardization of Clinical Testing (Nex-StoCT) workgroup[8] and the College of American Pathologists[9].

Many diagnostic cancer samples are preserved in formalin fixed paraffin embedded (FFPE) tissue blocks, containing fragmented or cross-linked DNA, with few whole genomes reported from FFPE samples to date. It has been suggested that if cancer tissue is not preserved appropriately (for example, snap frozen in addition to formalin fixed) that this could constitute willful destruction of evidence, necessitating that clear practice guidelines are generated to describe acceptable standard of care around tissue preservation for treatment-focused testing[1]. In response to this practical problem, new approaches are being developed to ensure optimal use of FFPE sections, such that sufficient information may be obtainable[10].

The availability of NGS has resulted in datasets ripe for interrogation and new insights. Companies are racing to provide panel tests, which interrogate hundreds of cancer genes, each gene included because it has been proven or hypothesised to be a cancer-causing or cancer-driving gene. This includes panels such as the Foundation Medicine T5a test[11]. If a potentially actionable aberration is detected by sequencing, for example, a mutation which is known or predicted to cause a non-functional (tumor suppressor) or activated (onco)-gene, then a recommendation may be made regarding the utility of a targeted therapy which may impact on that gene, or its associated pathway. The level of evidence underlying such a recommendation is variable[12]. Access to the right drug may be problematic and the chance or durability of response in that tumor type usually unknown. How should we validate potential actionable aberrations to aid in clinical trial design and choice of treatments for patients?

2. What constitutes an actionable aberration?

2.1 Human tumor cohort association studies

The molecular analysis of human tumors has the potential to unlock a series of molecular alerts or flags that may be predictive of drug response or resistance. In this setting, an actionable aberration is a molecular flag, which is underpinned by variable levels of evidence to suggest that a therapy targeting this aberration could be effective[12]. To date, molecular interrogation of cancer specimens has varied from analysis of expression of single genes or proteins by *in situ* hydridization or immunohistochemistry, or DNA sequencing of single genes (eg *KRAS* in lung cancer or colorectal cancer[4] or *BRAF* in melanoma or colorectal cancer through to DNA sequencing of up to several hundred genes (such as the Foundation One T5a test[11]). Analysis of the whole exome or whole genome is available but the interpretation is problematic and these are not approved to guide treatment, outside research studies.

In the research setting, massive parallel sequencing of multiple tumor genomes has produced a plethora of data, with some emergent themes. The Cancer Genome Atlas (TCGA) is on its way to providing comprehensive characterization of cancer genomes from 24 of the most common as well as nine of the most rare tumor types. Much of this work has already been published, with insights stretching from the analysis of cell of origin across cancer types[13] to

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detailed subsetting within cancer types eg for ovarian cancer[14], glioblastoma[15] and most recently, gastric cancer[16]. These data are valuable in helping us to understand the diverse biology of different tumors, for example, that genomic stability is a major determinant of subtype[13,16]. Similarly, the identification of a constellation of aberrations within a particular gene or pathway can indicate a therapeutic approach for a subset of cancers. For example, *PIK3CA* mutations were found in one of four subsets of gastric cancer[16]. This subset was defined by enrichment of high Epstein-Barr virus (EBV) burden, extensive DNA promoter methylation and 80% mutation rate in *PIK3CA*, suggesting that PI(3)-kinase inhibition should be examined in this patient group. Of interest, the *PIK3CA* mutations were more dispersed in EBV-positive gastric cancers, whereas in EBV-negative gastric cancers, *PIK3CA* mutations were localized in the kinase domain (exon 20) and present at a lower rate (3–42%) in the other three subtypes. The therapeutic implications of these findings remain to be determined.

The TCGA studies to date suggest that multiple novel cancer-associated genes remain to be discovered, particularly as new tumor types are analysed in depth[17]. Most cancer genes identified to date, appear to be altered in only 2–20% of cancers and will need to better understood, in order to be efficiently targeted in the clinic. Importantly, these studies of cancer genome associations are largely correlative and do not provide proof that a specific gene or pathway is indeed actionable. Pre-clinical models allowing functional interrogation can provide additional evidence that a target may be worth addressing in the clinic, as will be discussed in Sections 4 and 5.

3. Does the context of a molecular aberration matter?

An increasing number of distinct tumor types are being recognized in the clinic, as a result of newly defined molecular subsets. In order to design therapy targeted to specific tumor subsets, as well as to rare cancer types, many of which have limited treatment options[18], it is important to know whether a therapeutic outcome is informative from one tumor context to another. This requires consideration of histologic subtype, the gene or drug target involved, the type of molecular aberration (eg amplification versus activating mutation) and the molecular context, as will be discussed in Sections 3.1–3.3. Understanding this complexity, which cannot be encompassed in the clinical trial setting, lends weight to the need for pre-clinical models for proof of principle analysis, in order to underpin clinical trial directions.

3.1 Histologic tumor type

During the early stages of the National Cancer Institute's (NCI) Developmental Therapeutics Program (DTP) in the 1950's, initially only three transplanted rodent models were used (sarcoma, leukemia and carcinoma), hoping that they would be informative for a wide range of tumor types[19]. As that proved increasingly unlikely, other approaches were adopted including human xenografts and the NCI panel of 61 human tumor cell lines. Studies were also performed comparing whether cell line xenograft responses from one tumor type would be predictive for clinical outcomes of other tumor types in the clinic. These approaches yielded some clues but were largely disappointing[20].

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3.1.1 TP53 mutation, ubiquitous in some and variable in other tumor types— Mutations in the *TP53* tumor suppressor gene have been correlated with poor prognosis and poor treatment response in many cancers. The incidence of mutations in *TP53* varies by cancer type, ranging from near 100% in high-grade serous ovarian cancer (HGSC)[21], with much lower rates of mutation seen in some hematological malignancies, including follicular lymphoma or diffuse large B cell lymphoma. In HGSC, mutation of TP53 is an early event in tumorogenesis, which together with its ubiquitous nature in HGSC, may mean it is less likely to represent an actionable target. In contrast, this mutation in lymphoma is associated with relative drug resistance and may be used in a predictive fashion, to alter therapeutic decisions[22].

3.1.2 BRAF mutation in melanoma compared with colorectal cancer—The context in which a specific molecular vulnerability occurs (such as the tumor of origin) may influence its biologic behavior. Activating mutations of *BRAF (V600E)* occur in both melanoma and colorectal cancer. In *BRAF* mutant melanoma, the *BRAF* inhibitor, vemurafenib has shown impressive clinical benefit[23]. However, relative resistance is seen *BRAF (V600E)* mutant colorectal cancers treated with *BRAF* inhibitors. In contrast with melanoma, *BRAF (V600E)* inhibition in colorectal cancer is mediated by alternative signaling pathways including EGFR[24,25] or PI3K/AKT pathway activation[5], resulting in malignant growth. Potent synergy is seen with relevant inhibitor combinations in *in vitro* and *in vivo* in colon cancer models,[24,25] suggesting the synthetic lethality of combination therapies as a viable clinical strategy. Thus, the environment in which a mutation occurs, which may be specific to the original tumor type, may heavily influence the effect of a given molecular mutation[26].

3.1.3 ERBB2 amplification or over-expression in breast cancer compared with other cancer types—Molecular therapies are transforming the practice of oncology. In breast cancer, as a result of large scale, well organized clinical trials, treatment with trastuzumab, pertuzumab, lapatinib, TDM-1 and other therapies targeting ERBB2 (also known as human epidermal growth factor receptor 2 (HER2)), have established roles in adjuvant and metastatic disease, with clear survival advantages in ERBB2-amplified disease [3,27,28,29,30]. In gastric cancer, the ToGA trial has conclusively shown that ERBB2 amplification is a predictive marker for response to trastuzumab, with significant improvements in median overall and progression free survival with the addition of trastuzumab to chemotherapy[31]. ERBB2 gene amplification or protein overexpression has also been reported in 11% of esophageal squamous cell cancer[32] and in 18 – 35% of mucinous epithelial ovarian cancer, providing a potential actionable target for the management of these cancers[33].

3.2 The type of aberration in a validated target

3.2.1 ERBB2 activating mutation, rather than amplification—Since 2004, activating mutations have been identified in ERBB2, across multiple tumor types, including non-small cell lung cancer and mucinous ovarian cancer[34]. As these aberrations do not involve over-expression of the receptor, small molecule tyrosine kinase inhibitors acting on the intra-cellular domain would be most likely to be of use, rather than antibodies directed

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against the extra-cellular domain[34]. Thus, the choice of therapeutic strategy would be dictated by the aberration and although the mutation rate in each particular tumor type is low, clinical trials are needed to address whether the pipeline of ERBB2-directed TKIs could be of clinical utility for these patients.

3.2.2 ALK amplification, rather than ALK translocation—Molecular inhibitors of specific aberrancies may have broader efficacy in anomalies of the putative signaling pathway. The identification of Anaplastic lymphoma kinase (*ALK*) translocations as oncogenic drivers in non-small cell lung cancer, coupled with the rapid development of crizotinib, has dramatically altered the therapeutic landscape in this disease. Preclinical responses to crizotinib have been demonstrated in inflammatory breast cancer showing *ALK* amplifications (increased *ALK* copy number, as distinct from gene translocations) stimulating clinical trials evaluating ALK targeted therapeutics in patients with broader *ALK* abnormalities[35]. *ALK* amplifications are common events in esophageal cancers (where *ALK* translocations are not present), providing potential targets for selective inhibition as an attractive clinical strategy.

3.3 Molecular context

3.3.1 PARP inhibitor response in DNA-repair defective HGSC—PARP inhibitor efficacy is known to be dependent on the presence of a DNA repair defect in the homologous recombination pathway, such as seen with HGSC mutated for either *BRCA1* or *BRCA2*[36,37,38]. It was initially proposed that PARP inhibitor efficacy was mediated by effects on the Base Excision Repair pathway, however, Patel and colleagues showed impaired efficacy of PARP inhibition in HGSC cell lines in which non-homologous end-joining (NHEJ) had been disabled[39]. This suggested that NHEJ hyperactivation may be responsible for PARP inhibitor induced lethality in HR-incompetent cells. Thus, in the expected context, that of *BRCA1/2* mutant HR defective HGSC, PARP inhibitor action may be prevented by the lack of an essential collaborating pathway, that of intact, indeed, hyperactivated NHEJ.

In addition to better understanding the complexity of genetic alterations in multiple tumor types, we need to predict the implications of these alterations, in order to improve design of combination therapy studies. Indeed, we need a coordinated effort to map genetic dependences, understand feedback loops and recognize crosstalk circuits which might indicate successful combination therapeutic approaches, as simply trying two drugs which might be predicted to work together is proving to be inefficient[26]. Equally, the concept of embracing complexity and adopting a combination of systems biology methods and integrated analyses may be necessary[40]. This complexity requires rigorous analysis in improved pre-clinical models.

4. Tractable pre-clinical models for prioritizing targets

4.1 Tractable models of human cancer

In order to provide accurate prediction as to whether a particular molecular aberration is indeed actionable, models systems are required which accurately represent human disease

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and are reasonably stable from passage to passage such that repeat analysis over time yields the same result[41]. These models need to generate material suitable for a full range of molecular analyses, including selection experiments to determine the impact of genome-wide screens or drug library screens. As cancer cells evolve to evade whatever selective pressure they are placed under, for example, drug pressure, the ability to model response after multiple lines of therapy should be an essential component of a translational model, recapitulating the patient journey and allowing analysis of clonal evolution.

4.2 Long-established cancer cell lines

The pre-clinical use of carefully curated, genomically-annotated cancer cell lines can provide biologic insights which help to validate putative targets, including those identified by molecular analysis[42]. Cell culture systems however, have inherent insufficiencies undermining their validity as comprehensive models and have failed to reliably predict clinical responses, as demonstrated by the National Cancer Institute drug development program over the last several decades[43]. Selection during tissue culture over time, may artificially eradicate features of the host tumor, pertinent for faithful replication of drug response and may activate spurious cell signaling pathways[44,45]. Domcke and colleagues have recently showed that many HGSC cell lines fail to recapitulate the genomic features of this disease[46]. None of the five popular cell lines accounting for 90% of the relevant literature were considered good quality lines, with the two cell lines, SK-OV-3 and A2780, which account for 60% of the literature, considered poorly suited as models of HGSC.

Cancer cell lines show low fidelity compared with complex genetic and epigenetic abnormalities existing in human tumors, and lack the stromal and immune influence of the human tumor microenvironment. As demonstrated by drug testing, xenografts generated from long-established human cancer cell lines may show inconsistent therapeutic responses[47] and poor correlation with clinical outcomes, in comparison with human primary orthotopic tumor xenografts[48,20]. Whilst the judicious use of molecularly curated cancer cell lines may inform mechanistic evaluation, cell culture systems lack the capacity to comprehensively model malignant processes, underscoring the urgent requirement for improved model systems.

4.3 Patient-derived xenografts (PDX)

An increasingly accepted preclinical model producing translational advances, is that of the patient-derived xenograft (PDX)[2]. The transplantation of tumor obtained at the time of surgery or biopsy, unmanipulated, into recipient mice, generates *in vivo* models which are tractable, renewable and have massive potential for parallel, sequential and long-term therapy experiments (Figure 1). The ability to drive drug resistance, as happens in a patient, allows the comparison of tumor and circulating tumor DNA from plasma, in a way that is not possible in the clinic[49].

4.3.1 Terminology: PDX, Avatars and Super-Avatars—The development of genetically modified immunodeficient mice has allowed for the generation of tumor xenograft models in which patient tumor obtained at the time of surgery or biopsy can be transplanted directly into mice without any *in vitro* manipulation. This type of model is

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known as a PDX the methodology of which has recently been extensively reviewed[2]. Tumors that successfully engraft can then be serially transplanted into subsequent generations of mice, generating a renewable resource, which can be annotated in detail and used to study novel therapies. Tumors engraft typically 2–4 months after transplantation[2]; however many factors can influence successful engraftment including method of processing and mode of transplantation, the recipient murine strain used and the tumor type being transplanted[50]. Within the field of cancer research, PDX models have taken various forms and as many of these adaptations have not previously been described and defined, the authors here attempt for the first time to define subsets of PDX models. “Avatars” represent a subset of PDX models that have the distinction of being transplanted in an orthotopic location and have not been previously subjected to cancer therapeutics[50] (Figure 1). In contrast, we define “super-avatars” as models generated by co-transplantation of hematopoietic stem cells (HPSC) and patient tumor, via an orthotopic route, which offer the potential of studying novel immunotherapies, described in more detail below (Figure 1).

4.3.2 The generation of PDX models—Orthotopic models are some of the most clinically translatable models in oncology research, as they recapitulate aspects of the clinical disease that are not shared by cell lines. It is important to note that different types of tumors may have different requirements for optimal transplantation and some may be easier (HGSC) and some more difficult (ER-positive breast cancer) to transplant[2,49,51,52]. Many PDX models are established by transplanting tumor from the patient into immunodeficient mice via the subcutaneous route or other locations for improved engraftment and easy of injection. Using this approach many aspects of the tumor microenvironment may be lost with successive rounds of transplantation, including stromal cells (although the human stroma from the initial graft tends to be replaced with murine stroma which often takes on a very similar morphologic appearance to the initial human graft) and various aspects of the innate and adaptive immune system[53]. Some subcutaneous xenografts fail to progress or to metastasize with the same pattern as human disease and therefore do not reflect all patterns of tumor progression seen in patients[54]. Certain tumor types when injected orthotopically recapitulate the tumor microenvironment and better track patient disease progress. These tumor types include ovarian[51,55,56], breast[57–61], pancreatic[62–67], renal[68–72], non-small cell lung[73–76] and melanoma[77–79]. The use of orthotopic tumor transplantation has been shown by many groups to accurately recapitulate patient tumor in an *in vivo* setting, DeRose and colleagues demonstrated that breast cancer tumor grafts developed metastases with frequencies from 38% to 100% in sites corresponding to patient metastatic sites, recapitulating the original patient metastatic cascade[51,57,80]. For all PDX, it is important to note whether the starting material was derived from a chemotherapy-naive tumor (upon first diagnosis, prior to any treatment) or after specific lines of treatment. Paired PDX from the same patient, before and after systemic therapy, have been reported using tumor from breast cancer patients[52]. The generation of similar models in other cancer types could be very valuable for exploring clonal evolution in response to therapy, including with analysis of circulating tumor DNA (ctDNA) (Figure 2).

A range of PDX models derived from unmanipulated primary human solid tumors without intervening *in vitro* culture, have been successfully used to demonstrate utility in predicting

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efficacy of a range of therapeutic approaches (Table 1). In these studies, PDX were annotated for specific molecular biomarkers of relevance for the drug in question. Response to the novel targeted therapeutic was documented in the light of the molecular context and could be utilized to underpin clinical trial design.

4.3.3 Therapy response in PDX or Avatars reflects that seen in patients—

Chemotherapies are used to treat a wide variety of tumor types[81]. Platinum agents are capable of directly binding to DNA, causing adducts that lead to the formation of single and double strand DNA breaks during replication and translation[82]. Coordinated platinum salts, such as cisplatin and carboplatin, are the cornerstone, for example, of epithelial ovarian cancer treatment and are the components of first-line treatment in this tumor type. Sensitivity to platinum-based therapy in ovarian cancer is disease-defining and prognostic, as platinum resistance correlates with poor outcomes. As such, being able to predict sensitivity, and potentially identify alternative therapies early, can have a clinical impact. Two recent reports demonstrated that HGSC PDX faithfully recapitulated response to platinum when compared with the outcome of treatment of the patient: Topp and colleagues defined platinum response for subcutaneous HGSC PDX, with three of four platinum sensitive HGSC PDX containing DNA repair gene mutations, and the fourth being methylated for *BRCA1*, whereas in contrast, all three platinum refractory PDX overexpressed dominant oncogenes (such as *CCNE1*, *LIN28B* and/or *BCL2*)[49]. In keeping with this, Weroha and colleagues used nine HGSC intra-peritoneal Avatar models, treated with four rounds of carboplatin/paclitaxel. When compared to patient response, nine out of nine tumorgrafts demonstrated *in vivo* platinum response reflective of the patient's clinical response[51]. Patient Avatar models can be valuable tools in predicting which patients might benefit from the use of platinum-based therapies and more importantly, highlighting those for whom platinum might have limited potential, with the requirement of other therapies in the short-term.

In a breast cancer model, Zhang and colleagues established PDX models representing a variety of breast cancer subtypes, which were treated with single agent docetaxel, doxorubicin, or combined trastuzumab and lapatinib, depending on the treatment received by the patient from which the PDX was derived. In this report a significant association between the PDX and patient treatment response was observed, with 12 of the 13 PDX responses matching the patient's clinical response[52]. Garralda and colleagues, used Avatar models along with whole-exome sequencing analysis in order to inform the treatment of patients with advanced stage solid tumors, including colorectal cancer, glioblastoma, non-small cell lung cancer, melanoma and pancreatic cancer[83]. A total of 13 treatments were directed by genomic and/or PDX model data, with 11 of the 13 models response mimicking the patient response.

Finally, PDX/Avatars can be generated from tumor samples obtained from warm autopsy. The aim of this approach is to obtain multiple biopsies from different metastatic sites from the one patient at the time of treatment failure and then to directly compare similarities and differences between samples. As the immediate engraftment of all sites of procurement may be cost prohibitive, it is feasible to viably preserve the material, molecularly screen it and then determine which metastatic sites to engraft and compare functionally. This would

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address the issue of heterogeneity at the end of the patient journey, in direct comparison with PDX/Avatars generated from chemo-naive patients at the time of diagnostic surgery, prior to any cancer treatment, when tumor heterogeneity may be less of an issue. The fact that many studies report comparable outcomes from a PDX derived from a single tumor site, when compared with the patient response to treatment, suggests that tumor heterogeneity may not be such an issue at first diagnosis, at least for cancers such as HGSC[49,51].

5. Next-generation Avatars

5.1 Super-Avatars: co-engrafted *in vivo* models; human tumor cells and hematopoietic stem cells

PDX/Avatar approaches are gaining in applicability and becoming powerful tools for studying tumor biology and assessing novel therapeutic approaches in the preclinical setting. While these systems recapitulate many aspects of the tumor microenvironment, they preclude studying interactions between immune and cancer cells[84]. The knowledge that tumor cells have evolved complex mechanisms to evade immunological response has led to the development of many immuno-oncology targeted treatments[85]. In colorectal cancer, Old and colleagues showed that patients whose tumors were infiltrated by lymphocytes had a better chance of survival[86]. Subsequent work in other tumor types has sought to further understand and leverage the immune system's ability to recognize tumor cells, a concept known as immunosurveillance, and the immune system's ability to protect against tumor growth and metastasis, a process known as immunoediting[87]. There is an urgent need to develop models that allow us to characterize the interactions between immune and cancer cells in the tumor microenvironment.

The co-engraftment of hematopoietic stem cells (HPSC) and patient tumor presents new challenges, as engraftment of HPSC and patient tumor may occur at different rates (Figure 1). Successful co-engraftment depends on two main factors: isolation of the HPSC and the murine strain used. HPSC are adult stem cells capable of repopulating all the hematopoietic lineages *in vivo* and sustaining production of these cells for the life span of an individual[88]. Transplantation and xenograft repopulation assays are routinely achieved by isolation and re-injection of CD34+ cells, a cell-cell adhesion factor that also mediate the attachment of stem cells to bone marrow[89,90]. The strain of mouse used can also influence engraftment success; the development of three different murine strains with IL-2 receptor mutations, has increased rates of engraftment: NOD.Cg-$Prkdc^{scid}Il2rg^{tm1Wjl}$ (NSG mice), NODShi.Cg-$Prkdc^{scid}Il2rg^{tm1Sug}$ (NOG mice) and C;129S4-$Rag2^{tm1Flv}Il2rg^{tm1Flv}$ (BRG mice)[91,92]. Of these models, NSG lack the *IL-2* receptor, whilst NOG and BRG mice express a truncated *IL-2* receptor, resulting in all these models lacking cytokine responses and expressing defective NK cells[92]. Subsequent mouse strains, denoted MITRG and MISTRG have been generated to increase engraftment success. Human versions of genes encoding human M-CSF (*csf1*), human interleukin 3 (IL-3) and GM-CSF, and human thrombopoietin were generated as a transgenic model in respective mouse loci in *Rag2*–/– *Il2rg*–/– mice (MITRG mice). The resulting human cytokines support the development and function of monocytes, macrophages and NK cells derived from human fetal liver or adult CD34(+) progenitor cells co-injected into the mice[93]. MISTRG mice also bear a bacterial artificial chromosome

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(BAC) transgene encoding human SIRPα, which binds CD47 and the resulting signal suppresses phagocytosis of CD47-expressing cells, enabling mouse phagocytes to "tolerate" and not clear engrafted human cells. These models may prove very useful for studies of therapies targeting both the tumor and the host immune system.

Various groups have laid the ground-work for such studies through the generation of cancer models designed to examine the interaction between immune and tumor cells[54,94]. Bankert and colleagues investigated tumor-associated T cells present in the donor graft (patient-derived) and found that T-cells remained active following transplantation for seven days[54]. This model can be utilized to provide valuable information of the events leading to inactivation of T-cells in human ovarian tumors[54]. In a breast cancer model, Lehmann and colleagues co-engrafted mice with human umbilical cord hematopoietic stem cells and breast cancer cell lines[95]. This model offers a novel approach to generate completely humanized monoclonal antibodies for all tumor types, particularly those cancer subtypes with no currently available antibody therapy[95]. A more complete understanding of the complex interaction between the interaction of the cancer cells and the human immune is required in order to design novel immunotherapies that allow for strategies to leverage the immune systems ability to help target and attack primary and dissemination tumor cells[54].

5.2 Avoiding host-derived lymphoma in PDX/Avatar models

PDX represent a powerful experimental tool, however, several groups have observed the unanticipated formation of lymphomas[96–9849,51]. Although mechanisms leading to the not infrequent development of lymphoma are poorly understood and few studies have properly characterized the presence of lymphoma, T-cell activation is implicated[98]. Work by Ghanekar and colleagues demonstrated that the development of these lymphoma were Epstein-Barr Virus (EBV)-associated B-cell lymphomas, likely to result from reactivation of latent EBV introduced following transplantation into immunodeficient mice[96]. The SCID mouse strains commonly used for PDX development lack B and T lymphocytes, which allows for the unwanted, unregulated growth of lymphoma[97,99,100]. SCID mice with the addition of a Beige mutation, known as SCID/Beige, result in mice without B- and T-lymphocytes and defective natural killer (NK) cells [101]. NSG mice, which are lacking mature NK cell and mature B- and T-lymphoctes, have been used for PDX models and to study EBV[92,102]. Given the high prevalence of latent EBV infection in adults and the universal presence of B lymphocytes in solid tumors, this potentially cofounding process represents a potential pitfall of solid tumor xenografting[96]. The presence of B-cell lymphocytes can be addressed by having multiple (eg three) recipient mice implanted with each human tumor, as it is uncommon for more than one mouse per implanted tumor to develop lymphoma (Topp et al). Immunohistochemical straining of the tumor arising in each first passage recipient mouse for expression of pan-cytokeratin to confirm the epithelial origin of each tumorgraft, provides a simple way of differentiating engrafted tumors from solid lymphomas[49,51] Other approaches include the exclusion of leukocytes from source tissue or targeted therapies. Single-cell sorting by flow cytometry prior to transplantation would be the most stringent technique to remove any lymphocytes, as even the presence of a few B-cells can be sufficient to establish a lymphoma[96,103]. However, that requires solid tumor digestion, which may contribute to PDX drift. An alternative approach is the use of

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targeted treatment of recipient mice to prevent the development of lymphoma. Rituximab is a monoclonal antibody targeting the B-cell specific antigen CD20, which upon antibody binding signals for its destruction by NK cells[104]. The success of rituximab has spurred the development of other CD20 targeted agents such as ocerizumab, ofatumumab, and obinutuzumab[105]. Given that these mice are immunocompromised, the use of rituximab or any of these other targeted therapies would be helpful to reduce the occurrence of lymphoma.

5.3 Avatar models and their use in clinical trials

PDX provide a useful pre-clinical model to bridge the gap between *in vitro* studies and patient trials (Figure 3). These models remain histologically and genetically similar to their donor. They have also been shown to be predictive of clinical outcome and are being used for drug evaluation, biomarker identification, biological studies and personalized medicine studies[2]. Recent work has shown that response rates in PDX and Avatar models correlate with those observed in clinic, both for targeted agents and for classic cytotoxic drugs[49,51,52]. Thus, the potential for using these models for translational biomarkers development and directing individualize therapy in patients is increasingly recognized[2]. The generation of PDX can be established in co-clinical trials, which refers to trials conducted simultaneously in human patients and in PDX, in which both the patient and the PDX receive the same treatment[50]. This strategy is helpful as it permits the simultaneous assessment of drug response in both patient and mouse for correlative studies, identification of biomarkers of susceptibility and resistance, and investigation of novel therapies to address the emergences of resistance[2]. This may also aid in the investigation of exceptional responders exploring the molecular basis of why some tumors are very sensitive to certain treatments[106]. Such studies can be conducted in the PDX, even when little or no tissue remains in the source patient who experiences a dramatic response to therapy. In order for correlative models to aid the study of therapies in co-clinical trials, PDX can provide additional important *in vivo* functional information in parallel with *in vitro* assays such as the more commonly used spheroid and colony formation assays.

Co-clinical trials are often hampered by the fact that PDX and Avatar models are a costly and time consuming addition to any clinical trial, which are substantial barriers[50]. PDX can also be utilized to determine patient-specific targeted therapies, although outside clinical trials, we do not have sufficient evidence to suggest that study of a single PDX should guide patient treatment. This concept of personalized treatment presents with it challenges that must also be addressed at a conceptual level, as often only a small biopsy representing one portion of large heterogeneous tumor is transplanted. However, this may be mitigated by data that has demonstrated that PDX outcomes are broadly representative of patient outcomes[51,49]. Additionally, patients and mice may demonstrate different levels of drug toxicity, which may in part be due dosing schedules not always being fully translatable. Importantly, patients are often on additional medications and diets, which are not factored into PDX treatments[2,50]. When considering PDX with mutations in a particular gene of interest, it would be inappropriate for the study of only a few PDX representing a small spectrum of possible mutations, to be considered informative for all mutations in that gene.

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Despite these potential short-comings, Avatar mouse models have been used in pancreatic and non-small cell lung cancer to direct personalized cancer treatment[107,108]. In non-small cell lung cancer, Avatar models were used to test the efficacy of three common first-line chemotherapeutics, revealing that patients fall into different subgroups, with some showing sensitivity to various treatments and resistance to others[107]. Garralda and colleagues performed whole-exome sequencing on patients with advanced stage solid tumors and used Avatar models to test targeted therapies in the setting of patient-specific mutations. Using this strategy, six out of thirteen patients achieved durable remission[83]. Zhang and colleagues established 32 breast cancer PDX models representing a variety of breast cancer sub-types, which were all shown to be genomically consistent with the patient sample and demonstrated comparable treatment responses in both PDX and patient[52]. These early clinical results warrant further investigation. In conclusion, the use of PDX is proving to be a powerful tool to assess novel therapies in the co-clinical setting, however, in order to utilize the full potential, we must develop rigorous clinical trial platforms to allow Avatars to inform patient treatment. It is likely that Avatar models will contribute to development of personalized medicine as technology and our ability to reliably engraft tumors makes this approach more cost effective.

5.4 Generation of Avatar derivatives

PDX/Avatar cohorts, which have been molecular annotated and functionally analysed for response to standard and relevant novel therapies, become a valuable resource, for interrogation of specific hypotheses relevant for that histologic and molecular subtype. In addition to driving drug resistance and studying clonal evolution in tumor and ctDNA (matched tumor and plasma samples), PDX can be studied *ex vivo,* following a brief cellular digestion and fluorescent activated cell sorting (FACS) to generate a single cell suspension for analysis in drug screens, including drug library screens over 1–5 days (Figure 2). Cell lines can also be generated, although with *in vitro* culture the risk of generating culture artifact is ever present, requiring monitoring of cell line drift. Such PDX-derived cell lines could be used to support parallel *ex vivo* and *in vivo* PDX studies and are valuable additions to PDX models because of the depth of analysis performed on the PDX *in vivo*. Derivatives of PDX or PDX-derived cell lines (of limited passage number eg passage 10–50) can be generated and studied *in vitro* or *in vivo,* with the incorporation of additional modifications by siRNA, TALENS or CRISPR to enable specific gene editing, gene activation or reversible gene knockdown[42]. These robust technologies assist in the identification of critical genetic regulators of cellular processes. The incorporation of high-throughput library screens may facilitate powerful exploration of fundamental drivers of cellular machinery or drug effect[109,110]. By harnessing these techniques, well-annotated PDX and derivatives of specific molecular subtype may provide exquisite opportunities for studying relevant drug response and resistance mechanisms (Figure 2).

6. Conclusions

In conclusion, PDX provide a proof of principle opportunity, if carefully curated, to inform clinical trial design and improve outcomes for patients participating in clinical trials. While it is not practical for a PDX or Avatar to be developed in real time to direct most patient's

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front-line therapy, it may be possible for later lines of treatment. However, there is no doubt that much can be learned from the study of PDX. By employing these models, drugs brought to the clinic in clinical trials are more likely to be successful for that tumor type, due to improved treatment choice, based on better informed treatment-indication and knowledge of the drug resistance pattern likely to emerge. During treatment with one carefully chosen inhibitor, break-out resistance could be predicted, with the next inhibitor chosen as a preventive maintenance therapy to pre-empt that mode of resistance or as the next line therapy once relapse has emerged[111]. This approach can be trialed in PDX/Avatar models prior to being tested in the clinic. Indeed, in this way, more toxic combination therapies can be compared with less toxic sequential approaches, in the future with liquid biopsy of ctDNA surveillance for known drug resistance mechanisms.

Used in this way, PDX could allow prediction of likely mechanisms of drug resistance and in doing so, inform design of appropriate therapeutic strategies. By defining the rules of engagement in the fight against cancer evolution under treatment pressure, we should be better placed to prioritize treatment for our patients, who unlike PDX models, have far fewer chances to try multiple therapeutic options.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

WT wildtype

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Highlights

- Prioritizing potential therapeutic targets identified by genomics is challenging
- Well-annotated patient-derived xenografts (PDX) can be powerful patient surrogates
- PDX can be sampled serially and in parallel to reveal clonal evolution on treatment
- PDX could predict likely mechanisms of drug resistance to inform therapeutic strategy
- Challenges include high quality annotation and humanizing the murine immune system

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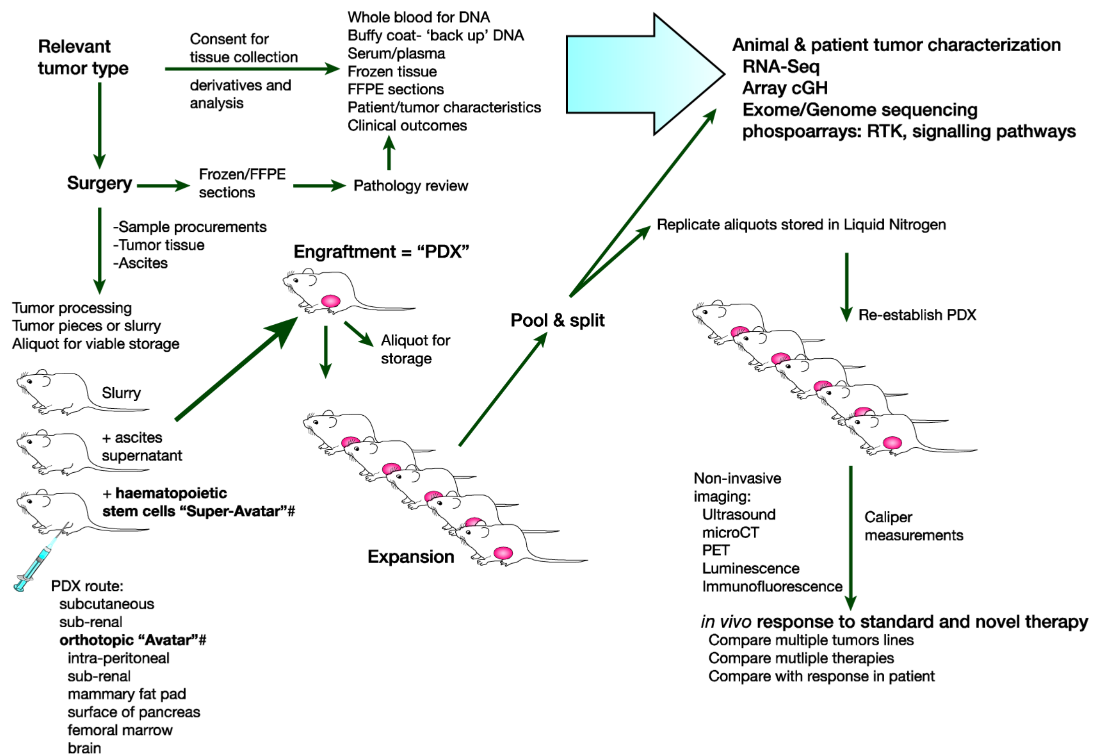

Figure 1. Flexibility of patient-derived xenograft models

By transplanting fresh, unmanipulated tumor at the time of biopsy or surgery into mice, stable patient-derived xenografts (PDX) can be generated with considerable similarity to the primary human cancer. Detailed functional and molecular analysis can be performed of the PDX can be compared with the primary human sample and with patient outcome. *In vivo* response to conventional and novel therapy can be performed in parallel for multiple drugs or sequentially, in order to drive drug resistance, as occurs in the clinic. A renewable resource can be generated by using viable freezings of minced tissue slurry. Orthotopic chemonaiive PDX can be referred to as "Avatars". Mice in which humanized immune reconstitution has also been performed can be termed "Super-Avatars". Receptor Tyrosine Kinase = RTK. Photoemission Tomography = PET.

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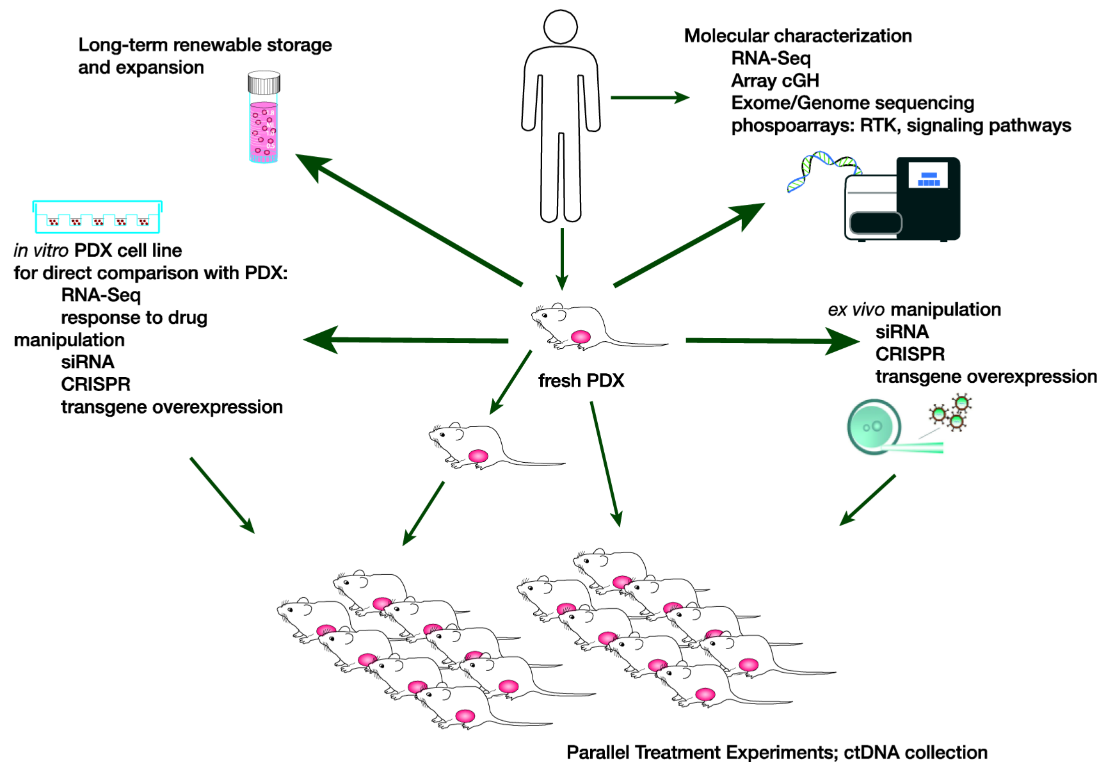

Figure 2. Derivatives generated from patient-derived xenografts

Once patient-derived xenografts (PDX) have been generated and annotated, additional derivatives can be generated, which further increase the utility of the original model, adding to its functionality. These include the generation of a cell line, which once compared molecularly with the baseline tumor and PDX, can be manipulated using techniques such as siRNA, CRISPR or transgene over-expression. Such cell lines can then be sub-cloned *in vivo* for further therapeutic analysis. Similar techniques can be applied directly to fresh PDX material using a short *ex vivo* process (24–48 hours *in vitro* culture). Parallel treatment experiments can be accompanied by surveillance for markers of drug resistance using ctDNA analysis. Receptor Tyrosine Kinase = RTK. Circulating tumor DNA = ctDNA.

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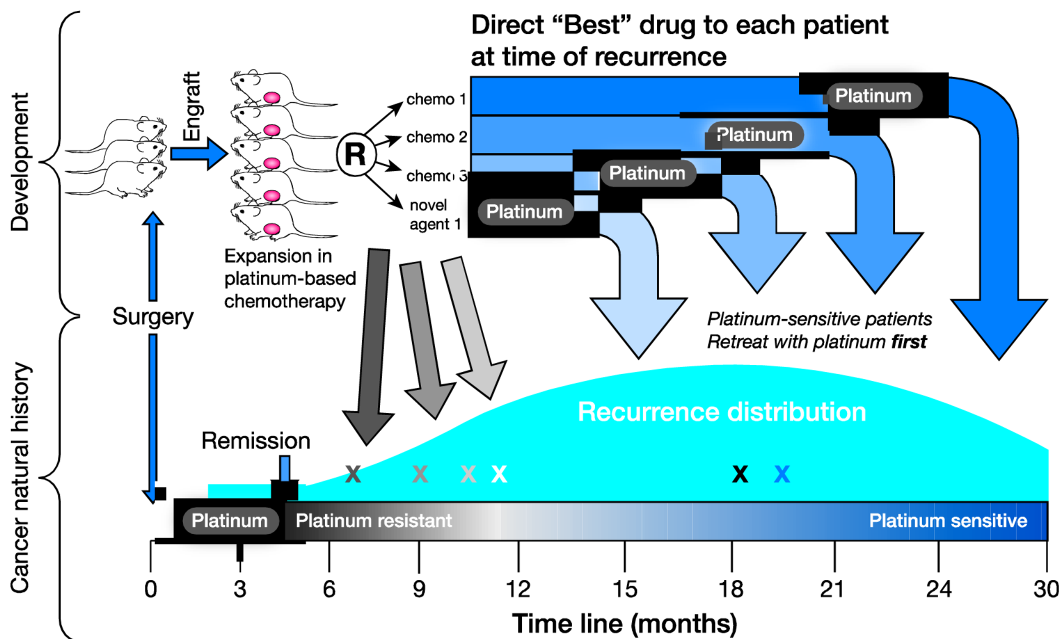

Figure 3. Avatar-directed trial schema

At the time of initial diagnosis, a chemonaiive patient-derived xenograft (PDX) can be generated and treated with the standard therapy for that tumor type (for example, a tumor-type for which platinum is a very important treatment, such as high-grade serous ovarian cancer). As clones emerge during platinum-based therapy, additional chemotherapeutics or novel drugs can be tested, in order to determine the best treatment for that patient upon relapse after standard therapy. chemotherapy = chemo.

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Table 1

Targeted therapeutic studies performed in PDX derived from primary patient samples with biomarker analysis

Examples of the use of PDX derived directly from primary solid human tumor with no intervening *in vitro* culture step, to study the efficacy of a targeted therapeutic in the setting of an exploratory or known biomarker.

Author	Tumor type	PDX type, site, mouse strain	Actionable target	n = independent PDX tested	Targeted therapy	Outcome
Fichtner *et. al.* Clin Cancer Res, 2008 [112]	NSCLC	subcutaneous NOD/SCID mice; nu/nu mice for drug testing	EGFR mutation, KRAS mutation	25 PDX	Cetuximab (EGFR mAb) and erlotinib (EGFR inhibitor)	>50% tumor growth inhibition: seen with cetuximab in 12 of 25 NSCLC xenografts, seen with erlotinib in 6 of 25. No correlation with EGFR mutations, K-ras mutations correlated with erlotinib resistance.
Zhang *et. al.* Clin Cancer Res, 2012 [113]	NSCLC	subcutaneous nu/nu mice	FGFR1 amplification	4 PDX	AZD4547 (selective small molecule FGFR inhibitor)	Potent tumor stasis/ regression in 4 of 5 FGFR1-amplified models
Zhang *et. al.* J Transl Med, 2013 [114]	NSCLC	subcutaneous SCID and nu/nu mice	*EGFR* activating mutation; *KRAS* mutation; *cMET gene* amplification; *FGFR1* amplification	10 PDX tested for EGFR, KRAS, cMET and FGFR1	Gefitinib (EGFR inhibitor)	*EGFR* activating mutation was most sensitive, *FGFR1* gene amplification were insensitive, *KRAS* mutation +/− *cMET* amplification was resistant (no model with cMET mutation alone)
Kortmann *et. al.* Clin Cancer Res, 2011 [115]	Ovarian (not noted as HGSC)	renal capsule, NOD-SCID mice	*BRCA2* germline mutation	2 PDX 1 s*BRCA1/2* WT 1 *BRCA2*m	Olaparib (PARP inhibitor)	*BRCA2* mutant PDX was sensitive to Olaparib
Das Thakur *et. al.* Nature, 2013 [116]	Melanoma	subcutaneous nu/nu mice	BRAF mutation (V600E)	2 PDX	Vemurafenib (BRAF inhibitor)	Demonstrated that resistant tumors show dependency on *BRAF* due to elevated BRAF(V600E) expression, and become drug-dependent for their continued proliferation; cessation of drug led to tumor regression

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Author	Tumor type	PDX type, site, mouse strain	Actionable target	n = independent PDX tested	Targeted therapy	Outcome
Marangoni *et. al.* Clin Cancer Res, 2007 [117]	Breast cancer	interscapular fat pad, Swiss nu/nu mice	HER2 amplification	2 PDX HER2 amplified	Trastuzumab (HER2 mAb)	One of two HER2-amplified xenografts responded to trastuzumab
Ma *et. al.* JCI, 2012 [118]	Breast cancer	mammary fat pad*, NOD-SCID mice	TNBC, p53 mutation	3 PDX 1 p53 WT 2 p53 mutant	Chk1 inhibitor +/− irinotecan	Combination therapy induced checkpoint bypass and apoptosis in p53 mutant tumors; inhibited tumor growth and prolonged survival of p53 mutant TNBC. (PDX cell line retrovirally infected with shp53)
Dave *et. al.* PLoS One, 2012 [119]	Breast cancer	mammary fat pad*, SCID Beige mice	TNBC, pSTAT3 expression	2 PDX	STAT3 inhibitor +/− docetaxel	Tumor growth and recurrence inhibited by combination in one chemoresistant PDX (high pSTAT3 expression) and not in one chemosensitive PDX; pSTAT3 expression reduced post STAT3 inhibitor
Oakes *et. al.* PNAS, 2012 [120]	Breast cancer	mammary fat pad*, NOD-SCID-IL2R$\gamma_c^{-/-}$ mice	BCL-2 expression	5 PDX variable expression of BCL-2	BH3 mimetic +/− docetaxel,	Improved tumor response and overall survival with combination for PDX with elevated levels of BCL-2
Vaillant *et. al.* Cancer Cell, 2013 [121]	Breast cancer	mammary fat pad*, NOD-SCID-IL2R$\gamma_c^{-/-}$ mice	ER positive, BCL-2 expression, pAKT expression	4 PDX variable expression of BCL-2	BH3 mimetic/tamoxifen +/− PI3K/mTOR inhibitor	BH3 mimetic improved tumor response to tamoxifen. Median survival was significantly prolonged with triple therapy compared to tamoxifen/ABT-737 in the PDX with highest expression of BCL-2
Julien *et. al.* Clin Cancer Res, 2012 [122]	CRC	subcutaneous Swiss nu/nu mice or rats	KRAS	52 PDX	Cetuximab (EGFR mAb)	25 PDX models responded to cetuximab. Improved survival for wild-type KRAS models (grouped) versus mutated KRAS models (grouped); 12/28 KRAS WT models did not respond to cetuximab.

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Author	Tumor type	PDX type, site, mouse strain	Actionable target	n = independent PDX tested	Targeted therapy	Outcome
Pawaskar *et. al.* Cancer Chemother Pharmacol, 2013 [123]	Pancreas cancer	subcutaneous CB-17 SCID mice	KRAS/Raf pathway, PI3 k-Akt-mTOR pathway	2 PDX	Sorafenib, (Raf, VEGFR and PDGF-b inhibitor) + everolimus (mTOR inhibitor)	Higher doses of single agent sorafenib and everolimus inhibited tumor growth; complete inhibition of tumor growth in combination
Rubio-Viqueira *et. al.* Clin Cancer Res, 2006 [124]	Pancreas cancer	subcutaneous nu/nu mice	EGFR, mTOR and Erk activation	14 PDX	Erlotinib (EGFR inhibitor), temsirolimus (mTOR inhibitor), and CI-1040 (Erk inhibitor)	Inhibition by CI-1040 seen with Erk activation (14%), 7% response rate to Temsirolimus, no Erlotinib responses
Cao *et. al.* Br J Cancer, 2009 [125]	Pancreas cancerContact	subcutaneous SCID mice; surface of pancreas*	PI3K/Akt/mTOR deregulation	5 PDX	NVP-BEZ235 (dual PI3K/mTOR inhibitor)	Suppression of phosphorylation of PKB/Akt. Chronic dosing produced modest tumor growth inhibition in three of five PDX models
Al-Ejeh *et. al.* Clin Cancer Res, 2014 [126]	Pancreas cancer	subcutaneous NOD-SCID-IL2R$\gamma_c^{-/-}$ and nu/nu mice	CHK1, EGFR	1 PDX	PF-477736 (CHK1 inhibitor), radioimmuno therapy (Lutetium-labeled anti-EGFR) and gemcitabine	Complete regression with triple combination therapy
Sivanand *et. al.* Sci Transl Med, 2012 [127]	Renal cancer	renal capsule* NOD/SCID mice (similar results for subcutaneous vs subrenal)	mTORC pathway	9 PDX	Dovitinib, sunitinib (multikinase inhibitors), sirolimus (mTORinhibi tor), erlotinib (EGFR inhibitor)	PDX retained sensitivities to sunitinib and (tem)sirolimus observed in the clinic and failed to respond to the control drug, erlotinib. Dovitinib more potently inhibited PDX than did sunitinib or sirolimus (tested in 1 PDX)
Su *et. al.* Molecular Cancer 2014 [128]	Renal cancer	subcutaneous NOD/SCID mice	microRNA (let-7d)	1 PDX	intratumoral injection of cholesterol-conjugated let-7d mimics	Tumor growth suppressed, tumor weight decreased. Number of metastatic colonies and the quantification of human-specific Alu-sequence in mouse lung were also reduced
Stewart E, *et al.* Cell Reports, 2014 [129]	Ewings Sarcoma	Femoral marrow* CD1 nu/nu mice	PARP-1	1 PDX	Veliparib, Olaparib, BMN-673 +/− irinotecan, or temozolomide	Complete remission for PARPi + chemotherapy in > 80% of mice

*orthotopic site