Studies of Cancer Heterogeneity Using PDX Models

5

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Abbreviations

ABC	Activated B-cell
AI	Allelic imbalance
BCR	B-cell receptor
CAF	Cancer-associated fibroblasts
CCC	Consensus clustering classification
CNA	Copy number alterations
DLBCL	Diffuse large B-cell lymphoma
ECM	Extracellular matrix
EGFR	Epidermal growth factor receptor
HNSCC	Head and neck squamous cell carcinomas
JUND	Jun D proto-oncogene
LBCL	Large B-cell lymphoma
PDXs	Patient-derived xenografts
TAM	Tumor-associated macrophages
TGFBR3	Transforming growth factor b receptor 3
TNBCs	Triple negative breast cancers
T_{reg}	Regulatory T-cell

Introduction

Preclinical cancer models are of paramount importance in the development of anticancer drugs. Xenograft models based on cultured cancer cell lines have played a key role in this process. Thus, the NCI-60 cancer cell line panel has been used for over 25 years for anticancer drug screening. As valuable a resource as the cell lines

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have been, their grafts have significant limitations, such as lack of predictive power in drug efficacy tests as they fail to mimic the enormous complexity of human cancers, including their tumor heterogeneity and tissue architecture. It is also not precisely known how these cell lines differ genomically and functionally from the primary cancer cells from which they were derived. Moreover, they lack clinical information, such as treatments administered, patient outcome, response to therapy and stage of disease at diagnosis, etc. To overcome such limitations, transplantable patient-derived xenografts (PDXs) have been generated in the last decade by engrafting fresh human tumor *tissue* into immunodeficient mice. These PDXs more closely mimic the patients' tumors with regard to tumor microenvironment, retaining interactions between cancer cells and stromal cells. They exhibit a high degree of genomic stability in comparison to their parental tumors, even after serial transplantation of tumor sections. As well, a PDX model significantly retains the heterogeneity of the tumor from which it is derived.

In this chapter, we review genomic, transcriptomic, and proteomic heterogeneities of PDX models, compared to parental tissues, in the light of current advances in research and understanding.

Genomic and Proteomic Heterogeneity of PDX Models

Intra-tumor heterogeneity is thought to stem from two factors: (1) cell autonomous, including genomic, transcriptomic, and proteomic heterogeneity and (2) non-cell autonomous, for example, stromal heterogeneity. Tumor heterogeneity has clinical implications for patient-specific responses to therapy and resistance to targeted therapy [1]. PDX models are capable of retaining tumor heterogeneity, so these models have clear advantages over traditional cell line-based models and are becoming the preferred tools in drug discovery and preclinical studies [2, 3].

Gaub et al. established seven PDX models in nude mice for human colonic tumors (from stages B1 to D) in order to study correlations between initial tumors and PDX models. They used allelotyping analysis to test 45 loci (on 18 chromosomes) on the seven original tumors and their sequential PDXs and scored retention of the genetic alterations present in the original tumors after xenografting. The original tumors showed chromosome profile instability between fragments of the same tumor in an allelic imbalance (AI) assay. After the xenografting, all the AIs were maintained in PDX models compared to the original tumors, and the maintenance of the genetic profiles of the tumors could be observed even after serial transplantation for up to 14 passages. These results proved that intra-tumor clonal heterogeneity was conserved in the PDX models of the seven colonic tumors [4].

In Landen et al.'s research, it was demonstrated that PDX models of ovarian cancer can also recapitulate the original tumor's heterogeneity. They examined oncogenic expression, proliferation, and response to chemotherapy and found that xenografts recapitulated the heterogeneity of tumor-initiating cells in the original patient tumor, although the stromal component was murine. The PDX models had similar oncogene expressions as the original tumor and responded to chemotherapy

in a similar manner as the patients from which the original tumors had been harvested [5].

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous B-cell cancer defined by signaling and survival pathways, multiple genetic alterations, and transcriptional classifications. Rodig et al. generated nine large B-cell lymphoma (LBCL) PDX models, including eight DLBCL and one plasmablastic lymphoma. They used whole-exome sequencing to identify mutations and chromosomal alterations and whole-transcriptome sequencing to classify cells of origin and consensus clustering classification (CCC) subtypes. Six of the eight DLBCL models were activated B-cell (ABC)-type tumors and exhibited ABC-associated mutations such as CARD11, CD79B, MYD88, and PIM1. The other two DLBCL models were germinal B-cell type and showed alterations of CREBBP, EZH2, and GNA13 and chromosomal translocations involving IgH and either BCL2 or MYC. Six of the eight DLBCL PDX models were B-cell receptor (BCR)-type tumors identified by CCC criteria, and they exhibited BCR selective surface immunoglobulin expression. The reflection of the transcriptional, genetic, and immune-phenotypic heterogeneity of primary DLBCL in PDX models indicates that PDX models for DLBCL are effective and faithful as reported for solid tumors [6].

PDX models have been developed for a few malignancies, including colonic [4, 7], ovarian [5], pancreatic [8], and breast cancers [9, 10], non-small cell lung cancers [11], as well as large B-cell lymphoma [6] and medulloblastoma [12]. Although these PDX models were shown to closely recapitulate the histology and gene expression patterns of the primary tumors, some genomic, transcriptomic, or proteomic differences were also observed between the PDXs and the patients' tumors.

In Fang et al.'s study, a collection of PDX models for hepatocellular carcinoma (HCC) was established. These models recapitulated the complexity of the original tumors, including gene expression profiles, mutational status, and DNA copy number alterations (CNA), with few differences found. For example, of the gene expression profiles, genes related to DNA replication and cell cycling were upregulated in the PDXs. They compared 286 HCC patient samples with 42 HCC PDX models and found copy number gains in the following genes: *PBX1* (76.2%), *PRCC* (76.2%), *ARNT* (61.9%), *BCL9* (59.5%), *MTDH* (52.4%), *COX6C* (52.4%), *ABL2* (50%), *MET* (42.9%), *CCND1* (16.7%), *FGF19* (14.3%), and losses of *AFF1* (76%), *RAP1GDS1* (71%), *WRN* (71.4%), *PCM1* (71.4%), *WHSC1L1* (66.7%), *RB1* (59.5%), *BRCA2* (57.1%), *CDKN2A* (57.1%), *CDH1* (50%), *CDKN2B* (45.2%), *TSC2* (38.1%), *SMAD4* (33.3%), *APC* (28.6%), *STK11* (26.2%), *WT1* (23.8%), *MLH1* (21.4%), *TNFAIP3* (21.4%), *PTEN* (19.1%), *CDKN2C* (16.7%), *ARID1A* (14.3%), and *TNFRSF14* (11.9%). The results suggest that oncogenes were enriched during the xenografting [13].

Mardis et al. established a panel of PDX models for human basal-like breast cancer and analyzed four DNA samples for one patient to get genomic information on peripheral blood, primary tumor, brain metastasis, and the xenograft derived from the primary tumor. Compared with the primary tumor, the metastasis exhibited enrichment for 20 shared mutations, a large deletion not present in the primary tumor and two de novo mutations. The PDX models retained all primary tumor mutations as

expected, while the mutation enrichment pattern of the PDX highly resembled that of the metastasis. They identified 50 novel somatic point mutations and small indels (insertion/deletion). The wide range of mutant allele frequencies displayed genetic heterogeneity in the cell population of the primary tumor. The mutation frequency range narrowed in the brain metastasis and PDX, which may indicate that the metastatic and xenografting processes selected for cells carrying a distinct subset of the primary tumor mutation repertoire. The overlap between the mutation frequency both changed in the metastatic and xenograft samples suggested that cellular selection during xenografting was similar to that during metastasis [14].

Differences between original tumors and PDX models have also been observed in head and neck squamous cell carcinomas (HNSCC). Grandis JR et al. compared the protein expressions of PDXs with those of HNSCCs and found that, whereas the majority of proteins were similarly expressed, 64 proteins were differentially expressed in the PDXs: 30 proteins showing increased expression, whereas 34 showed reduced expression. There were only six proteins, i.e., AKT, c-Myc, PR, BCL2, c-Kit, and HSP70, with more than half of the PDX models outside the expression range of primary HNSCCs. AKT, c-Myc, and PR showed increased expression in PDXs, whereas the expressions of BCL2, c-Kit, and HSP70 were decreased. This protein expression panel indicates that proteins associated with cell proliferation may be preferentially selected during the development of the xenografts [15].

Differences between original tumors and PDX models have been reported for several types of cancer, such as breast [14, 16, 17], colonic [18], and liver cancer [13] and head and neck squamous cell carcinoma [15]. The differences may be explained by the following theories: (1) In response to stress-inducing events, specific cells which had preference expression patterns could survive more easily than others, (2) the xenografts evolve dynamically in order to adapt to growth in different hosts [17], (3) the replacement of the human stroma with mouse stromal cells after engraftment, and (4) loss of non-transformed epithelial cells.

PDX Models Retain Cell-Autonomous Heterogeneity

Human tumor heterogeneity creates a complex microenvironment that enables cell growth, development of therapy resistance, and metastasis [19, 20]. Cell lines cultured from cancer samples which were collected decades ago are still used in laboratories, yet pronounced differences in molecular profiles have been found between commonly used ovarian cancer cell lines and high-grade serous ovarian cancer samples [21]. In vitro cell cultures lack the stroma and mesenchymal elements present in human tumors to generate the paracrine production of growth factors and signaling pathways necessary to support tumor proliferation and metastasis formation [22–24]. Continuous subculturing of cells and passaging with enzyme treatment used for in vitro cell maintenance may be selecting a genetically and phenotypically uniform cancer cell subclone that flourishes in the plastic dish of the laboratory setting which, however, lacks the heterogeneous microenvironment seen in human tumors [25].

Because in vitro cell cultures lack heterogeneity, researchers have investigated alternative models that more closely resemble human tumors. Xenograft models, generated by engrafting established cell lines in mice, are widely used by researchers, but the functional utility [26] and the accuracy of such conventional xenografts lacking the donor tumor heterogeneity and tumor microenvironment [27] have been questioned. For decades, preclinical research in malignancies has largely relied upon cloned cancer-derived cell lines and tumor xenografts derived from these cell lines. However, the cell lines used for translational research have disadvantages, as genetic and phenotypic alterations from serial passaging have resulted in expression profiles that are different from those of the original patient tumors. Preclinical models, such as cell line-based xenograft models, often fail to retain the diverse heterogeneity of human tumors and hence lack clinical predictive power.

Intra-tumoral heterogeneity plays an important role in driving the extent of drug response and the development of therapy resistance. The existence of multiple subclones in human tumors explains variable response rates to therapy, even within a single tumor mass, and the rapid emergence of drug resistance. For example, the presence of a minor KRAS-mutant clone can predict which colorectal cancer patients will develop resistance to epidermal growth factor receptor (EGFR)-targeted therapy [28]. Curtis et al. showed that breast cancer had at least ten distinct molecular subtypes with significant differences in disease outcome and responses to therapy [29]. There is an association between clonal diversity and drug resistance for at least some tumor types—notably ovarian [30] and esophageal [31]. Basal-like triple negative breast cancers (TNBCs) have previously been linked to shorter disease-free survival when compared to non-basal-like triple negative breast cancers and tend to be associated with higher clonal diversity [32]. Clearly, although more work has to be done, it seems likely that the clonal composition of tumors will have potential use for predicting disease outcome and informing treatment choice.

The above implications suggest that we need to advance toward using highly characterized tumor models, representative of the large variability of tumors in humans. Next-generation sequencing and single-cell sequencing studies have identified multiple genetically distinct clonal variants within a single human tumor, demonstrating the level of heterogeneity that exists in human tumors [33, 34]. Therefore, the models we choose to study the development of therapeutic drug resistance need to reflect (1) genetic variation and (2) the tumor microenvironment. Both factors will affect the sensitivity or response and eventual resistance of a tumor to therapy. There are several heterogeneity factors in a developing tumor, such as the presence of distinct clonal variants in the original tumor population, tumor-initiating subpopulations, and cells carrying “mutator” phenotypes that allow a tumor to develop therapy resistance. The better we model all these aspects of intra-tumoral heterogeneity, the more likely we are to capture the dynamic nature of resistance. In order to create better models of human cancers, PDX models have been developed. The PDX models, derived from patient tumor tissues as distinct from cultured cell lines, have, by virtue of recapitulating as much of the human variation as possible, emerged as a powerful technology showing better representation of the heterogeneity of tumors, and part of the human tumor microenvironment, with preservation of

cellular complexity, genetics, vascular, and stromal tumor architecture. PDX cancer models are likely best suited for (1) studying the emergence of multiple resistance mechanisms, (2) guiding therapeutic strategies to overcome relapsed tumors, and (3) using drug efficacy tests in the discovery and preclinical development of superior anticancer agents.

To examine whether PDXs are able to show varying responses of patients' tumors to chemotherapy, the Shanghai LIDE Biotech Company designed a reverse validation trial with S-1, a drug combination used for therapy of gastric cancers. The trial was performed on four gastric cancer samples (GAPF155, GAPF157, GAPF161, and GAPF187) that had successfully been engrafted into mice. Xenografts were propagated and treatment cohorts of 16 mice were generated for each implanted cancer specimen. When tumors reached an average volume of 200 mm^3, mice were randomized to receive either a placebo or S-1. Consistent with the treatment response of patients to S-1, as shown in Fig. 5.1, GAPF155 and GAPF157 were sensitive to S-1, whereas GAPF161 and GAPF187 were not sensitive to S-1. These results indicate that PDXs can reflect variable responses of patients' tumors to therapy.

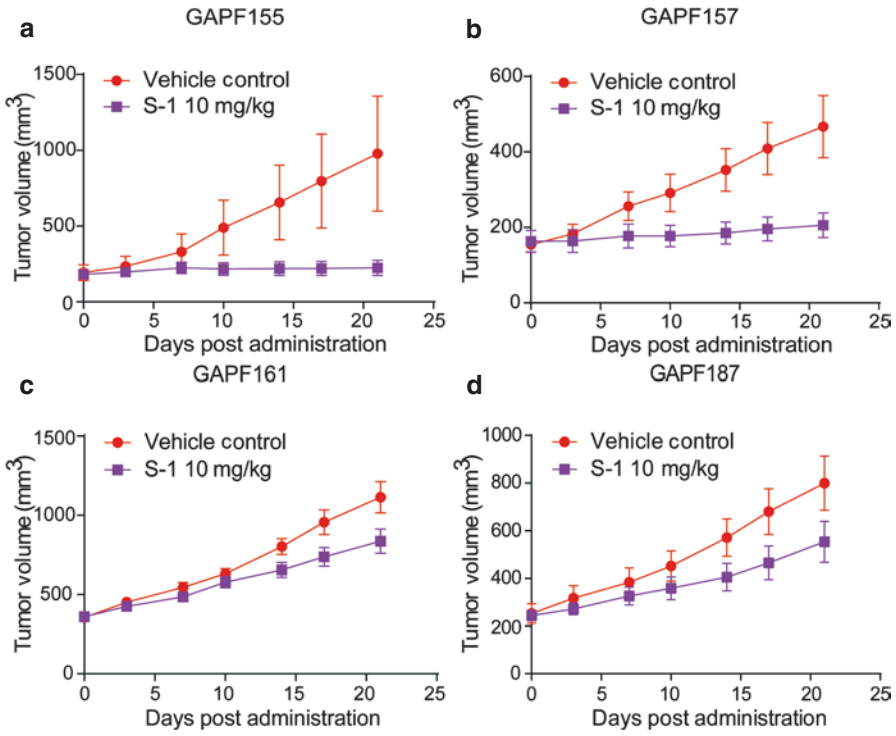

Fig. 5.1 Efficacy study of S-1 in four gastric PDX models. **a** and **b** show that GAPF155 and GAPF157 were sensitive to S-1; **c** and **d** show that GAPF161 and GAPF187 were not sensitive to S-1

PDX Models May Fail to Fully Account for Many Non-cell-Autonomous Drivers of Heterogeneity

PDXs are arguably the best models of tumor heterogeneity and therefore perhaps the most powerful tools for investigating tumor biology. Although PDX models maintain the genomic architecture, histology, and drug responsiveness of the original patients' tumors, the clonal profiles and tumor microenvironment of PDX tumors can change during their propagation in immunodeficient mice. Analysis of genome-wide variant allele frequencies in serial passages of PDX tumors showed that clonal selection occurs more frequently in initial engraftment steps than in propagation steps, but the detailed clonal dynamics differ depending on the various tumor samples of the same tumor type [35]. The clonal dynamics in PDX tumors is probably generated by selection acting on preexisting clones rather than by generation of new clones [36]. As a result, it is probable that the more aggressive clones become dominant in PDX tumors, and, in some cases, PDX models indeed showed the genomic and transcriptomic signature of metastatic and relapsed cancers [37]. These aggressive clones could be particularly important targets in cancer therapy.

As well, stromal and immune interactions in PDXs may be altered by cellular component deficiencies and interspecies compatibility in host models. However, the tumor microenvironment has long been known to play an essential role in tumor progression, and its role in drug response is becoming apparent [38, 39]. Aside from clonal dynamics driven by intrinsic differences in a cell's genetic or epigenetic background, intra-tumor heterogeneity can be influenced by tumor-extrinsic factors in the non-cell-autonomous compartment [40]. Cellular interactions with the extracellular matrix (ECM) can alter gene expression programs, drive differentiation, and profoundly alter cell behavior. As cancers develop, tight regulation of the ECM is lost and tissue architecture begins to degrade [38]. A study by Wang and colleagues [41] provides direct evidence that ECM-dependent signaling confers dynamic switching between transforming growth factor b receptor 3 (TGFBR3) and jun D proto-oncogene (JUND)-related expression signatures. ECM-driven oscillations between signaling pathways such as those described could have profound effects on propensity to malignancy. Furthermore, solid-state ECM interactions are necessary for cells to maintain stem cell properties, and regulated ECM helps maintain the stem cell niche [42]. In PDX models, Matrigel is often used to increase the engraftment efficiency; however, it is worth noting that this is a murine basement membrane extract, and suitable synthetic human alternatives are available. The presence of growth factors in Matrigel may favor the engraftment of one cell type over another. Finally, as ECM structure is tissue specific [42], researchers should consider the use of orthotopic transplantations where possible.

The tumor microenvironment is further characterized by an influx of stromal cells. Infiltrating cancer-associated fibroblasts (CAF) can often confer resistance to cytotoxic and targeted therapies [39]. Because of the high levels of CAF infiltrates seen in some tumor types, heterogeneity within their population would undoubtedly

confer differential properties to the tumor bulk. In PDX models, human stromal cells are gradually replaced by murine equivalents upon engraftment in the mouse, suggesting that implanted human cancer cells retain the ability to recruit murine accessory cells to their niche. However, it should be noted that some differences exist between ligand repertoires of human and murine fibroblasts. Clearly, stromal architecture and activity are mimicked in the murine host; however, it is currently unclear how this reflects human stroma with regard to supporting tumor growth and development.

The immune system also plays a crucial role in tumor progression, and perhaps it is the most obvious disadvantage in PDX models, because of engraftment into severely immune-deficient host animals. Tumor cells are broadly thought to be antigenic which emerge point mutations in coding exons in a developed tumor and result in a large repertoire of neoantigens. Targeting of these neoantigens can lead to significant $CD8^+$cytotoxic T-cell infiltration and tumor cell death. However, most tumors eventually progress and evade the immune system often through the dominant inhibitory effects of suppressive pathways (the so-called immune checkpoints such as CTLA-4/B7 and PD-1/PD-L1). This is supported by the prognostic value of the $CD8^+$ to $FOXP3^+$ (cytotoxic to regulatory T-cell, T_{reg}) ratio in many solid tumors and the recently reported clinical efficacy of a variety of checkpoint inhibitors [43, 44]. The proinflammatory microenvironment established by M1-polarized tumor-associated macrophages (TAM), $CD8^+$T-cells, NK cells, and others can lead to the recruitment of numerous immune-suppressive components. In addition, $CD4^+$T-cell and macrophage recruitment following intensive chemotherapy in breast cancer patients is associated with significantly reduced recurrence-free survival [44].

All in all, heterogeneity within a tumor is governed by both cell-autonomous (e.g., genetic and epigenetic heterogeneity) and non-cell-autonomous (e.g., stromal heterogeneity) drivers. Although PDXs can largely recapitulate the genomic architecture, histology, and drug responsiveness of human tumors, they may not fully account for heterogeneity in the tumor microenvironment. However, these models have substantial utility in basic and translational research in cancer biology, but study of stromal or immune drivers of tumor progression may be limited. Similarly, PDX models offer the ability to conduct in vivo and ex vivo patient-specific drug screens, but stromal contributions to treatment responses may be underrepresented.

References

1. Gao H, Korn JM, Ferretti S, Monahan JE, Wang Y, Singh M, et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nat Med. 2015;21(11):1318–25. doi:10.1038/nm.3954.
2. Sausville EA, Burger AM. Contributions of human tumor xenografts to anticancer drug development. Cancer Res. 2006;66(7):3351–3354. discussion 4. doi:10.1158/0008-5472.CAN-05-3627.
3. Siolas D, Hannon GJ. Patient-derived tumor xenografts: transforming clinical samples into mouse models. Cancer Res. 2013;73(17):5315–9. doi:10.1158/0008-5472.CAN-13-1069.

4. Guenot D, Guerin E, Aguillon-Romain S, Pencreach E, Schneider A, Neuville A, et al. Primary tumour genetic alterations and intra-tumoral heterogeneity are maintained in xenografts of human colon cancers showing chromosome instability. J Pathol. 2006;208(5):643–52. doi:10.1002/path.1936.
5. Dobbin ZC, Katre AA, Steg AD, Erickson BK, Shah MM, Alvarez RD, et al. Using heterogeneity of the patient-derived xenograft model to identify the chemoresistant population in ovarian cancer. Oncotarget. 2014;5(18):8750–64. doi:10.18632/oncotarget.2373.
6. Chapuy B, Cheng H, Watahiki A, Ducar MD, Tan Y, Chen L, et al. Diffuse large B-cell lymphoma patient-derived xenograft models capture the molecular and biological heterogeneity of the disease. Blood. 2016;127(18):2203–13. doi:10.1182/blood-2015-09-672352.
7. Seol HS, Kang HJ, Lee SI, Kim NE, Kim TI, Chun SM, et al. Development and characterization of a colon PDX model that reproduces drug responsiveness and the mutation profiles of its original tumor. Cancer Lett. 2014;345(1):56–64. doi:10.1016/j.canlet.2013.11.010.
8. Kim MP, Evans DB, Wang H, Abbruzzese JL, Fleming JB, Gallick GE. Generation of orthotopic and heterotopic human pancreatic cancer xenografts in immunodeficient mice. Nat Protoc. 2009;4(11):1670–80. doi:10.1038/nprot.2009.171.
9. Fleming JM, Miller TC, Meyer MJ, Ginsburg E, Vonderhaar BK. Local regulation of human breast xenograft models. J Cell Physiol. 2010;224(3):795–806. doi:10.1002/jcp.22190.
10. Petrillo LA, Wolf DM, Kapoun AM, Wang NJ, Barczak A, Xiao Y, et al. Xenografts faithfully recapitulate breast cancer-specific gene expression patterns of parent primary breast tumors. Breast Cancer Res Treat. 2012;135(3):913–22. doi:10.1007/s10549-012-2226-y.
11. Fichtner I, Rolff J, Soong R, Hoffmann J, Hammer S, Sommer A, et al. Establishment of patient-derived non-small cell lung cancer xenografts as models for the identification of predictive biomarkers. Clin Cancer Res. 2008;14(20):6456–68. doi:10.1158/1078-0432.CCR-08-0138.
12. Zhao X, Liu Z, Yu L, Zhang Y, Baxter P, Voicu H, et al. Global gene expression profiling confirms the molecular fidelity of primary tumor-based orthotopic xenograft mouse models of medulloblastoma. Neuro-Oncology. 2012;14(5):574–83. doi:10.1093/neuonc/nos061.
13. Gu Q, Zhang B, Sun H, Xu Q, Tan Y, Wang G, et al. Genomic characterization of a large panel of patient-derived hepatocellular carcinoma xenograft tumor models for preclinical development. Oncotarget. 2015;6(24):20160–76. doi:10.18632/oncotarget.3969.
14. Ding L, Ellis MJ, Li S, Larson DE, Chen K, Wallis JW, et al. Genome remodelling in a basal-like breast cancer metastasis and xenograft. Nature. 2010;464(7291):999–1005. doi:10.1038/nature08989.
15. Li H, Wheeler S, Park Y, Ju Z, Thomas SM, Fichera M, et al. Proteomic characterization of head and neck cancer patient-derived xenografts. Mol Cancer Res. 2016;14(3):278–86. doi:10.1158/1541-7786.MCR-15-0354.
16. Kabos P, Finlay-Schultz J, Li C, Kline E, Finlayson C, Wisell J, et al. Patient-derived luminal breast cancer xenografts retain hormone receptor heterogeneity and help define unique estrogen-dependent gene signatures. Breast Cancer Res Treat. 2012;135(2):415–32. doi:10.1007/s10549-012-2164-8.
17. Kanaya N, Somlo G, Wu J, Frankel P, Kai M, Liu X et al. Characterization of patient-derived tumor xenografts (PDXs) as models for estrogen receptor positive (ER+HER2− and ER+HER2+) breast cancers. J Steroid Biochem Mol Biol. 2016. doi:10.1016/j.jsbmb.2016.05.001.
18. Burgenske DM, Monsma DJ, Dylewski D, Scott SB, Sayfie AD, Kim DG, et al. Establishment of genetically diverse patient-derived xenografts of colorectal cancer. Am J Cancer Res. 2014;4(6):824–37.
19. Lengyel E. Ovarian cancer development and metastasis. Am J Pathol. 2010;177(3):1053–64. doi:10.2353/ajpath.2010.100105.
20. Lengyel E, Burdette JE, Kenny HA, Matei D, Pilrose J, Haluska P, et al. Epithelial ovarian cancer experimental models. Oncogene. 2014;33(28):3619–33. doi:10.1038/onc.2013.321.
21. Domcke S, Sinha R, Levine DA, Sander C, Schultz N. Evaluating cell lines as tumour models by comparison of genomic profiles. Nat Commun. 2013;4:2126. doi:10.1038/ncomms3126.

22. Rasmussen AA, Cullen KJ. Paracrine/autocrine regulation of breast cancer by the insulin-like growth factors. Breast Cancer Res Treat. 1998;47(3):219–33.
23. van Roozendaal CE, Gillis AJ, Klijn JG, van Ooijen B, Claassen CJ, Eggermont AM, et al. Loss of imprinting of IGF2 and not H19 in breast cancer, adjacent normal tissue and derived fibroblast cultures. FEBS Lett. 1998;437(1–2):107–11.
24. Vermeulen L, De Sousa EMF, van der Heijden M, Cameron K, de Jong JH, Borovski T, et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. Nat Cell Biol. 2010;12(5):468–76. doi:10.1038/ncb2048.
25. Williams SA, Anderson WC, Santaguida MT, Dylla SJ. Patient-derived xenografts, the cancer stem cell paradigm, and cancer pathobiology in the 21st century. Lab Invest. 2013;93(9):970–82. doi:10.1038/labinvest.2013.92.
26. Scott CL, Becker MA, Haluska P, Samimi G. Patient-derived xenograft models to improve targeted therapy in epithelial ovarian cancer treatment. Front Oncol. 2013;3:295. doi:10.3389/fonc.2013.00295.
27. Stordal B, Timms K, Farrelly A, Gallagher D, Busschots S, Renaud M, et al. BRCA1/2 mutation analysis in 41 ovarian cell lines reveals only one functionally deleterious BRCA1 mutation. Mol Oncol. 2013;7(3):567–79. doi:10.1016/j.molonc.2012.12.007.
28. Diaz Jr LA, Williams RT, Wu J, Kinde I, Hecht JR, Berlin J, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature. 2012;486(7404):537–40. doi:10.1038/nature11219.
29. Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, Dunning MJ, et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature. 2012;486(7403):346–52. doi:10.1038/nature10983.
30. Bashashati A, Ha G, Tone A, Ding J, Prentice LM, Roth A, et al. Distinct evolutionary trajectories of primary high-grade serous ovarian cancers revealed through spatial mutational profiling. J Pathol. 2013;231(1):21–34. doi:10.1002/path.4230.
31. Maley CC, Galipeau PC, Finley JC, Wongsurawat VJ, Li X, Sanchez CA, et al. Genetic clonal diversity predicts progression to esophageal adenocarcinoma. Nat Genet. 2006;38(4):468–73. doi:10.1038/ng1768.
32. Shah SP, Roth A, Goya R, Oloumi A, Ha G, Zhao Y, et al. The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature. 2012;486(7403):395–9. doi:10.1038/nature10933.
33. Anderson K, Lutz C, van Delft FW, Bateman CM, Guo Y, Colman SM, et al. Genetic variegation of clonal architecture and propagating cells in leukaemia. Nature. 2011;469(7330):356–61. doi:10.1038/nature09650.
34. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012;366(10):883–92. doi:10.1056/NEJMoa1113205.
35. Eirew P, Steif A, Khattra J, Ha G, Yap D, Farahani H, et al. Dynamics of genomic clones in breast cancer patient xenografts at single-cell resolution. Nature. 2015;518(7539):422–6. doi:10.1038/nature13952.
36. Aparicio S, Hidalgo M, Kung AL. Examining the utility of patient-derived xenograft mouse models. Nat Rev Cancer. 2015;15(5):311–6. doi:10.1038/nrc3944.
37. Rosfjord E, Lucas J, Li G, Gerber HP. Advances in patient-derived tumor xenografts: from target identification to predicting clinical response rates in oncology. Biochem Pharmacol. 2014;91(2):135–43. doi:10.1016/j.bcp.2014.06.008.
38. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med. 2013;19(11):1423–37. doi:10.1038/nm.3394.
39. Straussman R, Morikawa T, Shee K, Barzily-Rokni M, Qian ZR, Du J, et al. Tumour microenvironment elicits innate resistance to RAF inhibitors through HGF secretion. Nature. 2012;487(7408):500–4. doi:10.1038/nature11183.
40. Michor F, Weaver VM. Understanding tissue context influences on intratumour heterogeneity. Nat Cell Biol. 2014;16(4):301–2. doi:10.1038/ncb2942.

41. Wang CC, Bajikar SS, Jamal L, Atkins KA, Janes KA. A time- and matrix-dependent TGFBR3-JUND-KRT5 regulatory circuit in single breast epithelial cells and basal-like premalignancies. Nat Cell Biol. 2014;16(4):345–56. doi:10.1038/ncb2930.
42. Cassidy JW. Nanotechnology in the regeneration of complex tissues. Bone Tissue Reg Insights. 2014;5:25–35. doi:10.4137/BTRI.S12331.
43. Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. Nature. 2014;515(7528):577–81. doi:10.1038/nature13988.
44. Schmidt M, Bohm D, von Torne C, Steiner E, Puhl A, Pilch H, et al. The humoral immune system has a key prognostic impact in node-negative breast cancer. Cancer Res. 2008;68(13):5405–13. doi:10.1158/0008-5472.CAN-07-5206.