

Patient-derived xenograft (PDX) models in basic and translational breast cancer research

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Abstract Patient-derived xenograft (PDX) models of a growing spectrum of cancers are rapidly supplanting long-established traditional cell lines as preferred models for conducting basic and translational preclinical research. In breast cancer, to complement the now curated collection of approximately 45 long-established human breast cancer cell lines, a newly formed consortium of academic laboratories, currently from Europe, Australia, and North America, herein summarizes data on over 500 stably transplantable PDX models representing all three clinical subtypes of breast cancer (ER+, HER2+, and “Triple-negative” (TNBC)). Many of these models are well-characterized with respect to genomic, transcriptomic, and proteomic features, metastatic behavior, and treatment response to a variety of standard-of-care and experimental therapeutics. These stably transplantable PDX

lines are generally available for dissemination to laboratories conducting translational research, and contact information for each collection is provided. This review summarizes current experiences related to PDX generation across participating groups, efforts to develop data standards for annotation and dissemination of patient clinical information that does not compromise patient privacy, efforts to develop complementary data standards for annotation of PDX characteristics and biology, and progress toward “credentialing” of PDX models as surrogates to represent individual patients for use in pre-clinical and co-clinical translational research. In addition, this review highlights important unresolved questions, as well as current limitations, that have hampered more efficient generation of PDX lines and more rapid adoption of PDX use in translational breast cancer research.

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1 Introduction

Human breast cancer is now recognized, not as a single disease, but as a heterogeneous collection of diseases characterized by diversity in histology, genomic alterations, gene expression, metastatic behavior, and treatment responses [1–7]. In addition to heterogeneity between tumors across the patient population (or between two primary tumors in a single patient), recent data have also demonstrated considerable intra-tumoral heterogeneity (that is, between cells within a single tumor and between a primary tumor and its associated metastases in a single patient). This degree of heterogeneity is a significant hindrance for making effective treatment decisions and begs for the development of personalized approaches to therapy based on the specific biology of an individual and their unique tumor [8]. With respect to basic and translational research, the existence of disease heterogeneity, both within and among breast cancers, also presents significant challenges to generation and use of relevant preclinical models that represent the full spectrum of breast disease [9, 10].

If our ultimate goal is to offer each breast cancer patient an individualized treatment plan tailored to her (or his) specific tumor and progression status, it will be essential to define fully the molecular and cellular heterogeneity within and among the tumor subtypes, and indeed within each patient's tumor, and relate these differences to clinical behavior. Specifically, these characteristics need to be linked to metastatic behavior and differential treatment response, the lethal aspects of breast cancer, in order to “personalize” effective treatment. Patient-derived xenograft (PDX) models hold high promise as a discovery and validation platform, particularly as a unified collection across multiple institutions, for meeting this daunting challenge.

1.1 The problem of inter-tumoral heterogeneity

Clinically, breast cancers are divided essentially into three subtypes: (1) those that express the estrogen receptor alpha (ER+) (which typically also express the progesterone receptor (PR+)), (2) those that are genetically amplified for and/or overexpress *ERBB2* (HER2+) (encoding a member of the epidermal growth factor receptor family of tyrosine kinases), and (3) those that express none of these three markers (termed “triple negative” breast cancer (TNBC)) [1–4]. While clinically useful, molecularly targeted therapies exist for ER+ breast cancer (e.g., selective estrogen receptor modulators (SERMs),

aromatase inhibitors (AIs), selective estrogen receptor downregulators (SERDs)) [11] and HER2+ breast cancer (e.g., trastuzumab, lapatinib) [12], there are currently no approved targeted therapies for TNBC. Current treatment of TNBC entails surgery coupled with radiotherapy and/or chemotherapy (most often taxane- or anthracycline-based, with platinum-based agents emerging as promising first-line therapies (e.g., [13])) in either the neoadjuvant (before surgery) or adjuvant (after surgery) settings.

With the development of RNA expression array technology over 15 years ago, more detailed molecular classification of breast cancer became possible. In a landmark analysis of global RNA expression, five intrinsic molecular subtypes of breast cancer were proposed: luminal A (ER+, with signatures consistent with lower proliferation rates that can be correlated with comparatively lower Ki67 immunostaining), luminal B (ER+, with signatures consistent with elevated proliferation that can be correlated with comparatively higher Ki67 immunostaining), basal-like (predominantly TNBC), HER2-enriched, and normal breast-like. Each molecular subtype correlated with unique clinical behavior including differences in overall survival, patterns of metastasis, and response to treatment [4–7]. Subsequently, the claudin-low subtype was identified. Claudin-low tumors are predominantly TNBC, with comparatively low expression of claudins 3, 4, and 7 [14], enrichment for mesenchymal markers [5], and enrichment of a stem cell-like ($CD44^+/CD24^{\text{Neg/Low}}$) gene expression signature [5, 15, 16].

Within the last 5 years, detailed genomic analyses of breast cancers by several groups including the ICGC, TCGA, and METABRIC consortia [17–21] have shown that additional molecular subtypes associated with distinct survival trends can be distinguished, such as the 4% of breast cancers that are ER+ with complex amplifications around 11q [18] and those showing differential survival associated with *PIK3CA* mutation [22, 23]. TNBC has been found to constitute at least two and perhaps more, distinct biological subgroups based on integrated genomics [24]—a genetically quiescent, *PIK3CA* mutation-containing, intermediate-good prognosis group (about 25% of TNBC) that groups more with ER+ cancers (a proportion express AR) of the IntClust4 subtype and the remainder which are almost universally mutated for *TP53* and have unstable genomes, a basal epithelial gene expression signature type, and poor prognosis with early relapse. Some TNBC gene expression studies have suggested TNBC could be subdivided further into four or more distinct subgroups [25–28].

1.2 The problem of intra-tumoral heterogeneity

Pathologists have long appreciated heterogeneity in both histology and biomarker expression within a given tumor. For example, in ER+ tumors, not all cells express the receptor. Clinically, ER+ breast cancers can be qualified as such even

if only 1% of cells express detectable levels of ER protein, though some low-expressing tumors may behave as ER-negative tumors in response to targeted therapies [29, 30]. Similarly, HER2+ tumors can show regional variation in expression by immunohistochemistry and copy number per cell by fluorescence *in situ* hybridization, with positivity clinically defined as 10% of cells positive (CAP Guidelines) [12, 31, 32].

Although clonal structure has been appreciated as a defining feature of cancers for several decades [33], methods for understanding clonal structure in solid epithelial cancers have only recently advanced. Until very recently, the existence of genetically distinct subpopulations within tumors, and within metastatic cell populations, was not appreciated (see [34–36] and references therein). With the development of computational approaches to infer clonal structure from bulk tumor sequence data (e.g., [20, 37–41]), as well as methods for single-cell DNA and RNA sequencing (e.g., [42–48]), breast cancers are now known to contain multiple genetically distinct subclones [49, 50]. Primary TNBCs have been shown to be clonally diverse among patients with the same stage/grade of tumor. Further work suggests that distinct subclones may, in some instances, be capable of interacting with one another to maintain homeostatic balance between clonal populations and promote tumor growth [51].

1.3 Patient-derived breast cancer xenografts as potentially powerful tools for preclinical and translational research

In theory, if PDX models can represent the full spectrum of heterogeneity of breast cancers in the population and can be fully “credentialed” to retain the critical molecular and biological properties of their tumor of origin, these models would then represent exceptionally powerful tools for translational research. In large part, their potential power lies in the fact that they have been demonstrated to be biologically stable (generally) and, as such, are renewable indefinitely. Thus, PDX models can be interrogated in greater depth both biologically and molecularly than any given patient sample is likely to be (unless very large). Further, PDX can be challenged with as many candidate therapeutics, or treatment regimens, as desired in a relatively short time frame versus what can be accomplished in the clinic. In contrast, the tumor of origin (and patient) can only be challenged sequentially with one treatment regimen at a time and only at significant risk of potentially lethal or debilitating side effects.

Herein, we garner the collective experience of a new international consortium of breast cancer PDX developers to review the state of the art in the field, to outline open questions remaining to be addressed, and to summarize the utility, limitations, and future promise of breast cancer PDX models in translational research.

2 The state of the art in breast cancer PDX modeling

2.1 Patient-derived xenograft models that represent all three clinically defined breast cancer subtypes have been established by various groups

In addition to the need to represent the heterogeneity of breast cancers as completely and accurately as possible, breast cancer is heavily influenced by the tumor microenvironment, making *in vivo* models desirable for studying complex processes like tumor metastasis and response to therapy. Efforts to establish stably transplantable xenograft lines directly from patients into immunocompromised mice have been going on for decades. Unfortunately, while initial take rates (i.e., initial outgrowths directly from patients) can be quite high (~40–90%) [52–54], success rates of generating stably transplantable xenografts (generally, but not uniformly, defined as PDX with the ability to be serially transplanted ≥ 3 passages in mice (variously termed “passage 2 (P2),” with P0 being the initial transplant from mouse to human, or “transplant generation 3 (TG3)”) (Tables 1 and 3) from early efforts were comparatively low, in the 10% range overall [52, 53, 55–64]. As a consequence, few stably transplantable models were established for dissemination to the research community from this earlier work.

More recently, with newly developed immunodeficient host mouse models [65] and modified transplantation conditions, overall stable take rates have now reached in excess of 20% on a routine basis (e.g., [13, 37, 54, 66–75]). Further, a concerted effort is being made to develop these collections of stable, well-characterized, PDX lines as quality-controlled, renewable tissue resources for distribution to the research community. A list of PDX collections available for dissemination, along with the patient/tumor population characteristics from which they were derived, is presented in Table 1; contact information for each collection is listed in Table 2. In addition to collections resident in academic institutions, several commercial entities retain PDX collections of their own, consisting of PDX lines either generated in-house or licensed from academic institutions. These commercial collections are not discussed here.

Taken in aggregate, the academic institutions participating in this consortium have developed 537 individual PDX lines, representing over 500 unique patients. With respect to clinical subtypes represented, 56% of patients yielding PDX lines had TNBC, with 36% of patients having ER+ cancer. Patients with HER2+ breast cancer are significantly underrepresented in these collections, representing only 8% of patients yielding PDX models, due to the combined facts that they represent only ~10–15% of all breast cancers and show a lower overall take

Table 1 PDX collection summary

Academic institution	Number of patients represented	Number of independent PDXs available	Clinical subtypes (by patient)				Tumor source(s)	
			Estrogen receptor-positive (including HER2+)	HER2-positive only	"Triple negative"	No data	Primary	Pleural effusion
Baylor College of Medicine (USA)	42	51	10 ^a	5	26 ^b	1	37	2
Bellvitge Institute for Biomedical Research (Spain)	11	11	3 ^c	0	8 ^d	0	3	0
British Columbia Cancer Agency (Canada)	40	40	13	6	19	1	24	4
Ecole Polytechnique Federale De Lausanne (Switzerland)	2	2	1	0	1 ^e	0	2	0
Institut Bergonié (France)	4	4	3	0	0	0	4	0
Institut Curie (France)	121	121	54	9	58 ^f	0	111	2
The Jackson Laboratory (JAX) (USA)	29	32	7	1	20	1	22	0
MD Anderson Cancer Center (USA)—Merle-Bernstam	26	29	13	0	13	0	24	0
University of Colorado Anschutz Medical Campus (USA)	25	25	11	1	12	1	20	2
University of Manchester Institute of Cancer Sciences (UK)	20	20	11	1	8	0	13	3
University of Michigan (USA)	20	21	3	3	14	0	15	5
University of Utah - Huntsman Cancer Institute (USA)	16	20	6	2	8 ^c	0	7	9
Walter and Eliza Hall Institute of Medical Research (WEHI) (Australia)	59	62	19 ^g	6	34 ⁱ	0	58	0
Washington University (USA)	68	73	27	4	37	0	33	0
Total	506	537	181	39	281	5	401	28
Percent of total	36	36	8	56	1		13	
Tumor source(s)								
Academic institution	Other metastatic site	Not specified	Race/ethnicity (by patient)				Not reported	
			Caucasian	Hispanic	African American	Continental African	Asian	
Baylor College of Medicine (USA)	1	0	10	21	9	0	0	2
Bellvitge Institute for Biomedical Research (Spain)	0	0	11	0	0	0	0	0
British Columbia Cancer Agency (Canada)	10	0	0	0	0	0	0	40
Ecole Polytechnique Federale De Lausanne (Switzerland)	0	0	2	0	0	0	0	0
Institut Bergonié (France)	0	0	0	0	0	0	0	4
Institut Curie (France)	8	0	0	0	0	0	0	121
The Jackson Laboratory (JAX) (USA)	4	3	21	1	0	0	2	5
MD Anderson Cancer Center (USA)—Merle-Bernstam	5	0	10	2	13	0	1	0
MD Anderson Cancer Center (USA)—Piwnica-Worms	1	0	12	3	6	0	2	0
University of Colorado Anschutz Medical Campus (USA)	1	0	0	0	0	0	0	25
University of Manchester Institute of Cancer Sciences (UK)	0	0	18	0	0	0	0	2
University of Michigan (USA)	1	0	6	0	4	9	0	1
University of Utah - Huntsman Cancer Institute (USA)	3	0	14	2	0	0	0	0
Walter and Eliza Hall Institute of Medical Research (WEHI) (Australia)	1	0	0	0	0	0	0	59
Washington University (USA)	40	0	50	0	18	0	0	0
Total	75	3	154	29	59	9	5	259
Percent of total	30	30	6	6	10	2	1	51

^aTwo BRCA1; one BRCA2), ^bFour BRCA1, ^cOne BRCA1, one metaplastic, two metaplastic mixed ductal), ^dOne BRCA2, four BRCA1, four BRCA2, four metaplastic, ^eDefined as "stable" at passage 2, ^hAll treatment naïve, ⁱFour BRCA1; one BRCA2, ^jOne BRCA1; one BRCA2, ^kEight BRCA1; one BRCA2; two metaplastic

rate than TNBC (which also accounts for 10–15% of all breast cancers, but shows a higher take rate) (Table 3).

Given the clinical observation that patients of different ethnic and racial backgrounds can have distinct treatment responses and disease outcomes, an important consideration is the ethnic and racial distribution of patients yielding PDX lines. In general, the diversity of PDX lines in a given collection represents the diversity in the associated patient population from which they were derived. While Caucasian women are most heavily represented across these collections, there are now a number of PDX lines representing African American and Hispanic women (Table 1). However, only a few PDX lines represent patients of Asian or African descent, and no PDX lines represent indigenous populations (e.g., Native Americans), or male breast cancer patients, among others.

Of note, the PDX lines representing Continental African patients from Ghana (Wicha et al., unpublished) were developed using primary tumor samples that had been viably frozen and shipped on dry ice prior to transplantation using a recently tested cryopreservation strategy (Table 3) (Lewis et al., unpublished), thus demonstrating that it should be possible to develop PDX tissue resources representing patients even from remote and underserved areas in the world where no active PDX development efforts may exist.

Finally, it is critical to recognize that the collections included in this review are exclusively from academic groups in Europe, Australia, and North America. Going forward, it will be important to include additional collections from other geographic regions that undoubtedly will have better representation of the patient demographics of the regions in which they function. Researchers and clinicians interested in participating in this International Breast Cancer Patient-Derived Xenograft Consortium should contact the corresponding author (M.T. Lewis) to be added to the mailing list.

3 Open questions regarding generation and use of breast PDX models

Detailed protocols currently used for generation of patient-derived xenograft (PDX) models of breast cancer have been published recently by De Rose et al. and Zhang et al. [54, 72] and will not be discussed in detail here. Similarly, the advantages and limitations of PDX models relative to clinical samples and established cell lines have also been discussed in detail elsewhere [76–79]. For the purposes of this review, we will briefly restate and highlight some of these strengths and limitations, but in the context of particular unresolved questions and issues currently being addressed by the breast PDX community.

Table 2 Contact information for PDX collections

Academic institution	Contact name and e-mail
Baylor College of Medicine (USA)	Michael Lewis (mtlewis@bcm.edu) Lacey Dobrolecki (dobrolec@bcm.edu)
Bellvitge Institute for Biomedical Research (Spain)	Eva Gonzalez-Suarez (egsuarez@idibell.cat)
British Columbia Cancer Agency (Canada)	Samuel Aparicio (saparicio@bccrc.ca)
Ecole Polytechnique Federale De Lausanne (Switzerland)	Cathrin Brisken (cathrin.brisken@epfl.ch)
Institut Bergonié (France)	Richard Iggo (r.iggo@bordeaux.unicancer.fr)
Institut Curie (France)	Elisabetta Marangoni (elisabetta.marangoni@curie.fr)
The Jackson Laboratory (JAX) (USA)	Carol Bult (Carol.Bult@jax.org) Susie Airhart (Susie.Airhart@jax.org)
MD Anderson Cancer Center (USA)—Meric-Bernstam	Funda Meric-Bernstam (fmeric@mdanderson.org)
MD Anderson Cancer Center (USA)—Piwnica-Worms	Helen Piwnica-Worms (hpiwnica-worms@mdanderson.org)
University of Colorado Anschutz Medical Campus (USA)	Carol Sartorius (Carol.Sartorius@ucdenver.edu)
University of Manchester Institute of Cancer Sciences (UK)	Peter Kabos (Peter.Kabos@ucdenver.edu)
University of Michigan (USA)	Robert Clarke (Robert.Clarke@manchester.ac.uk)
University of Utah—Huntsman Cancer Institute (USA)	Denis Alferez (denis.alferez@manchester.ac.uk)
Walter and Eliza Hall Institute of Medical Research (WEHI) (Australia)	Max Wicha (mwicha@med.umich.edu)
Washington University (USA)	Geoffrey Lindeman (lindeman@wehi.edu.au) Jane Visvader (visvader@wehi.edu.au) Shunqiang Li (shunqiangli@wustl.edu)

3.1 Are PDX really any better than xenografts made using long-established cell lines?

A number of immortalized or transformed cell lines have been established and extensively characterized over the last several decades, notably the MCF series, the MDA series, and more recently the SUM series (see [80–82] and references therein).

Table 3 Transplant conditions

Table 3 (continued)

^aDefined as "stable" through passage 3

^bDefined as “stable” through passage 2

² Five pending marker data

²Includes two TNBCs that converted to HER2+ upon passage

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Extensive efforts have been made to correlate gene expression, genomic copy number changes, and mutations with growth characteristics and therapeutic responses [83–86]. Unfortunately, while all of these cell lines grow *in vitro* under tissue culture conditions, only a percentage of these will grow when transplanted as cell line xenografts, and a fewer will form metastases despite many of them being derived from pleural effusions and ascites [87–91]. Thus, a large proportion of the studies aimed at translational endpoints using cell lines have been conducted *in vitro*, rather than *in vivo* as cell line xenografts.

It is now appreciated that gene expression patterns under two-dimensional *in vitro* culture conditions can be quite different than expression patterns observed under three-dimensional culture conditions, or when cell lines are grown as xenografts in immunocompromised mice [92]. Similarly, drug sensitivity under varied conditions can be different for a given cell line [93]. Indeed, in transcriptome studies of clinical samples versus established cancer cell lines, cell lines clustered together regardless of the tissue of origin, rather than clustering with the clinical samples they were intended to model [94]. Given these differences, it is perhaps to be expected that cell line-based studies have failed to translate clinically with high frequency and thus may not be suitable to address many clinical questions [8, 95]. In contrast, PDX lines are, by their nature, established and maintained *in vivo* and have been shown to retain a remarkable degree of biological, histological, genomic, transcriptomic, and biomarker fidelity with their tumors of origin (see below).

Most established human breast cancer cell lines have been maintained *in vitro* over several decades and in many different laboratories. Long-term culture has, in several cases, been associated with extensive clonal selection and loss of heterogeneity [94, 96]. Further, inconsistent handling as well as both inadvertent and deliberate selection are known to have led to genetic drift such that a multitude of sublines of individual parental lines now exist. As such, isolates of a given cell line (e.g., MCF7) can vary considerably from laboratory to laboratory with respect to genomics and gene expression [97, 98]. Each subline can thus possess its own unique attributes (invasive vs. not, metastatic vs. not, adherent vs. not, drug resistant vs. sensitive, etc.). Further, since the tumor of origin from which a given cell line was derived cannot be studied in most cases, and only limited clinically relevant data were collected, there is typically no way to evaluate what the original mutation spectrum in the patient was or what the clinical behavior of the tumor of origin actually were. Taken together, these shortcomings limit the utility of established breast cancer cell lines for predictive/correlative studies. However, these shortcomings do not necessarily impinge on the use of established cell lines to investigate basic biological mechanisms. In contrast, PDXs show behavioral, genomic, transcriptomic, and proteomic stability over at least 15 transplant generations in mice [54]. These attributes can be quality controlled from inception of the model,

and in many cases, this can be done with direct comparison with the clinically annotated tumor of origin.

Finally, unlike the vast majority of long-established human breast cancer cell lines, a majority of PDX lines were established from primary tumors rather than tumor cells derived from pleural effusions, ascites, or other metastatic sites. Thus, in perhaps an important way, PDX lines may serve to complement existing cell lines rather than supplant them, depending on the question(s) being asked.

3.2 To what degree do PDX models truly recapitulate the biology of the tumor of origin in the patient, particularly with respect to treatment response?

One of the main open questions relates to whether PDX models retain the intra-tumoral heterogeneity of the tumor of origin. While many PDXs appear to retain the heterogeneity of the parental tumor of origin, loss of heterogeneity, or a “bottlenecking” clonal selection upon transplantation, has been observed in others [44]. An example of such selection came from comparative genomic sequence analysis of a primary tumor, a patient-matched brain metastasis, and a PDX model derived from the primary tumor [37]. As expected, the metastatic lesion retained mutations found in the primary tumor but also possessed *de novo* mutations and deletions not observed in the primary tumor. Also, as expected, the PDX derived from the primary tumor retained the primary tumor mutations. Unexpectedly, the PDX showed the mutation spectrum found in the metastasis indicating that the metastatic subclone was present within the primary tumor and that it was this aggressive subclone that grew as a PDX. Thus, it is critical to compare the tumor of origin with its related PDX line as carefully as possible, whenever possible, to ensure accurate recapitulation of as much patient/tumor biology as possible, including tumor heterogeneity.

As recounted above, the inability to relate a cell line to its patient of origin is one of the primary places where long-established cell lines fall short. Thus, cell lines simply cannot be used for this purpose. In contrast, PDX models, and the tumors from which they were derived, have been compared directly at multiple levels in several studies. At the histological level, several studies have demonstrated that PDXs are virtually indistinguishable from the tumor of origin, including H&E-stained sections, as well as by immunostaining for biomarkers such as ER, PR, HER2, Ki67 positivity, etc. At the genomic level, PDXs show similar genomic rearrangements, copy number alterations, mutation profiles, and variant allele frequencies [71–73, 99, 100], observed in the tumor of origin. These results demonstrate not only stability of the PDX when transplanted from one species to another but also transplantability of clonal heterogeneity in many cases.

At the transcriptome level, PDXs generally show remarkable fidelity with respect to mRNA expression profiles

(RNAseq) [101, 73]. While proteomic comparisons between PDXs and their respective tumors of origin have not yet been completed, as stated previously, both transcriptomic and proteomic (RPPA) expression patterns have been shown to be remarkably stable across transplantation generations [54].

With respect to metastatic behavior, this is an open and active area of investigation across several groups. In general, PDXs have been shown to produce circulating tumor cells, as well as disseminated micrometastases and macrometastases to several distant sites ([71, 102, 54, 103] and Miragaya et al., unpublished). When evaluated for fidelity with the metastatic behavior of the tumor of origin, PDXs showed comparable metastatic site specificity ([71] and Miragaya et al., unpublished). As expected, PDX models are generally more metastatic in more immunocompromised hosts (e.g., larger metastatic nodules in non-obese diabetic (NOD) severe combined immunodeficiency disorder (SCID) gamma (NSG) mice vs. NOD/SCID; ALW, unpublished data). This could be due to faster tumor growth and/or more permissive colonization of the distant sites. These observations warrant further investigation to test the generality of these initial findings and to test the degree of influence of the immune system in the metastatic process.

3.3 What is the status of patient-PDX “credentialing” for relevance of PDX models in therapeutic studies?

Among the most critical issues to be addressed is whether PDX models respond to a given treatment in a manner similar to the tumor of origin in the patient. If so, PDX models should serve not only as relevant as experimental models but also as valuable translational research tools, especially if ultimately shown to have predictive value clinically. Again, this is a very active area of investigation in several groups. However, no “treatment standards” are yet available, largely due to the lack of comparative pharmacokinetic and pharmacodynamic data that would allow researchers to translate directly between PDX evaluation and clinical evaluation.

In preclinical treatment studies conducted to date, PDXs showed comparable responses to the tumor in the patient of origin when treated with comparable therapeutic agents. An early study evaluating seven xenografts showed an observed concordance of response between patient tumor and corresponding PDX of 71% versus a statistically expected concordance of 47% [68]. However, sample size was limited for direct comparison due to the types of treatment used clinically (adjuvant or post-radiation chemotherapy) in this patient cohort that could not easily be recapitulated in mice; thus, statistical significance was not quite reached using this sample size ($\kappa = 0.46, P = 0.08$). In a later study, a majority of xenograft lines tested also showed qualitatively identical treatment responses as the corresponding patient treated with a similar or identical agent in the neoadjuvant (before surgery) setting, and statistical significance was achieved using this increased

sample size [54]. Overall concordance of responses was ~92% ($K = 0.75, P = 0.003$), and there was a significant association between the xenograft and patient-derived results (Fisher exact test, $P = 0.04$). While both studies were relatively small, taken together, PDXs do recapitulate tumor responses seen clinically with good efficiency. Importantly, at least with respect to these selected standard-of-care agents, and under the specific conditions tested, this recapitulation does not appear to be influenced by the immunodeficiency of the host mice used. Thus, PDX models and associated data should serve as useful predictors of response in patients with at least some agents under some conditions. However, this hypothesis remains to be tested in a rigorous manner in both retrospective and prospective (co-)clinical trials.

3.4 Which immunocompromised mouse model should be used for most efficient PDX generation while still retaining the highest possible fidelity with the human disease?

A variety of immunocompromised, or immunodeficient, mouse models now exist that can be used as transplantation hosts to develop breast cancer PDX models (Tables 3 and 4). While all mouse models listed are capable of generating PDX models under various transplant conditions, and using various sample types, a consensus has yet to be reached as to the “best” host to use. Thus, choice of immunocompromised host remains largely a question of investigator preference until rigorous head-to-head studies are conducted, and a large enough body of data is available for multivariate analysis. Such data need to be related not only to the various transplantation conditions being employed but also to the characteristics of the underlying patient populations, and tumor types, being evaluated in order for meaningful conclusions to be drawn.

3.4.1 Athymic nude mice

Until approximately a decade ago, the most commonly used immunocompromised mouse model used for generation of breast cancer xenografts was the athymic “nude” mouse [104–106], which is homozygous for loss-of-function mutation of the *Foxn1* gene (encoding the forkhead box N1 transcription factor) (a.k.a. *nu*, *Hfh11*) [107, 108]. The *Foxn1* gene is essential for the development of the thymus and some ectodermal derivatives, including hair follicles (hence the “nude” phenotype), and leads to loss of functional T- and B-cells. The mice retain functional natural killer (NK) cells, macrophages, and antigen-presenting cells (APCs).

To support growth of estrogen-dependent breast cancer cell lines, athymic mice require supplementation with estradiol due to low endogenous levels of circulating estrogen [109–112]. The same appears to be true for other

Table 4 Host mouse strains used by institution

Institution	Host mouse strain abbreviation	Full strain designation	Vendor	Stock number
Baylor College of Medicine	NSG	NOD.Cg-Prkdc ^{scid} Il2rg ^{tm1Wjl} /SzJ	JAX	005557
	SCID/Bg	CB17.Cg-Prkdc ^{scid} Lyst ^{bg-J} /Crl	CR	250
Bellvitge Institute for Biomedical Research IDIBELL (Spain)	NSG	NOD.Cg-Prkdc ^{scid} Il2rg ^{tm1Wjl} /SzJ	JAX via CR	005557
	NOD/SCID	NOD.CB17-Prkdc ^{scid} /J	JAX via CR	001303
British Columbia Cancer Agency (Canada)	Nude	Athymic nude—Foxn1 ^{nu}	Harlan/Envigo	
	NSG	NOD.Cg-Prkdc ^{scid} Il2rg ^{tm1Wjl} /SzJ	JAX	005557
Ecole Polytechnique Federale De Lausanne (Switzerland)	NRG	NOD.Cg-Rag1 ^{tm1Mom} Il2rg ^{tm1Wjl} /SzJ	JAX	007799
	NSG	NOD.Cg-Prkdc ^{scid} Il2rg ^{tm1Wjl} /SzJ	JAX	005557
Institut Bergonié (France)	NSG	NOD.Cg-Prkdc ^{scid} Il2rg ^{tm1Wjl} /SzJ	JAX; bred in house	005557
Institut Curie (France)	Swiss Nude	Crl:NU(Ico)-Foxn1nu	CR	
The Jackson Laboratory (JAX) (USA)	NSG	NOD.Cg-Prkdc ^{scid} Il2rg ^{tm1Wjl} /SzJ	JAX	005557
MD Anderson Cancer Center (USA)—Meric-Bernstam	Nude	Athymic nude—Foxn1 ^{nu}	MDACC Colony	NA
MD Anderson Cancer Center (USA)—Piwnica-Worms	NOD/SCID	NOD.CB17-Prkdc ^{scid} /NcrCrl	CR	394
University of Colorado Anschutz Medical Campus (USA)	NOD/SCID	NOD.CB17-Prkdc ^{scid} /J	JAX	001303
	NSG	NOD.Cg-Prkdc ^{scid} Il2rg ^{tm1Wjl} /SzJ	JAX; bred in house	005557
University of Manchester Institute of Cancer Sciences (UK)	NSG	NOD.Cg-Prkdc ^{scid} Il2rg ^{tm1Wjl} /SzJ	JAX via CR	005557
University of Michigan (USA)	NSG	NOD.CB17-Prkdc ^{scid} /J	JAX; bred in house	005557;
	NOD/SCID	NOD.Cg-Prkdc ^{scid} Il2rg ^{tm1Wjl} /SzJ		001303
University of Utah Huntsman—Cancer Institute (USA)	NOD/SCID	NOD.CB17-Prkdc ^{scid} /J	JAX	001303
Walter and Eliza Hall Institute of Medical Research (WEHI) (Australia)	NSG	NOD.Cg-Prkdc ^{scid} Il2rg ^{tm1Wjl} /SzJ	JAX; bred in house	005557
Washington University (USA)	NSG	NOD.Cg-Prkdc ^{scid} Il2rg ^{tm1Wjl} /SzJ	JAX	005557

immunocompromised mice—even if they may have higher estrogen levels, cell lines only grow when the mice are supplemented with exogenous estradiol. While historical rates of generating stable PDX lines have been relatively low using nude mice, alternative transplantation methods (e.g., subcutaneous transplantation of tumor fragments into the intra-scapular fat pad or flank) do appear to allow efficient PDX establishment (Table 3) ([68, 113, 114], Gomez-Miragaya et al., unpublished).

3.4.2 Rag1/Rag2 mice

Rag recombinase-deficient (*Rag1* and/or *Rag2*) mice have been used sporadically for xenograft studies, mainly for generating cell line xenografts. However, these mice have not been used extensively for attempts to generate PDX lines (see Table 3). Like athymic nude mice, B- and T-cell function is abrogated [115] due to loss of function of the recombinases required for the somatic recombination of antibody chains and mature T-cell receptors. *Rag* knockout mice may be a useful alternative for some treatment response studies using anthracycline-based or other DNA-damaging

therapies because the consequences of *Rag* mutations are relatively specific to the hematopoietic system. As such, these mice can tolerate higher levels of DNA damage than mice carrying the SCID mutation (*Prkdc*, which encodes a ubiquitous DNA repair enzyme; see below) [116].

3.4.3 SCID mice

Most of the major breakthroughs made in efficient generation of PDX lines have been made using newer, more immunocompromised, mouse models. These include SCID mice which carry a mutation of the protein kinase, DNA-activated, catalytic polypeptide (*Prkdc*) gene encoding a protein kinase required for somatic variable, diversity, joining (VDJ) region recombination of antibody chains and T-cell receptors, as well as for DNA repair. Such mice show B- and T-cell deficiency, but background-dependent leakiness does occur. SCID mice retain cellular immunity [117–121]. The SCID mutation is generally used in combination with other mutations that further cripple the immune system for xenograft work. However, SCID mice remain prone to premature death,

due in part to their high prevalence of spontaneous T-cell lymphomas [121].

3.4.4 SCID/Beige mice

Combination of the *Beige* (*Bg*) mutation with the SCID mutation has been shown to enhance the take rate of human leukemias and other cell types, including breast cancers [122–125, 54]. In addition to B- and T-cell deficiencies, disruption of *Bg* results in a lysosomal trafficking defect and eliminates NK cell function, but leads to a ~3-fold increase in the number of macrophages relative to the parental wild-type mice [126, 127, 122, 123]. Macrophages are essential for normal mammary gland development. As such, this increase in macrophage content may be advantageous [128, 129]. Further, recent data showing promotion of tumor invasion and metastasis by immature myeloid cells of the macrophage lineage may account for some of the advantages of this immunocompromised background [130]. Use of this genetic background allows stable take rates in excess of 20% under various conditions, but successful transplantation in this background is enhanced significantly by estradiol supplementation (Table 3) [54], likely by an ER-alpha-mediated stimulation of bone marrow-derived monocytes [54, 131].

3.4.5 NOD/SCID mice

A major advance for the generation of PDX models came after genetic introgression of the NOD mutation into the SCID background, which compromises cellular immunity, via impaired function of NK cells, APCs, and macrophage cells. While impaired, presence of NK cells and macrophages in athymic and NOD/SCID mice can lead to elimination of tumor cells over time [132–134]. Use of this background has allowed take rates in excess of 20% on a routine basis (Table 3) (see [71, 135, 69, 136] and Gomez-Miragaya et al., unpublished; Piwnica-Worms et al., unpublished).

3.4.6 NOG and NSG mice

Development of the NOD/SCID background has continued with the addition of IL2-receptor gamma truncation/disruption mutations (“NOG” or “NSG” mice, respectively) which compromises the mouse immune system further by impairing cytokine signaling involved in innate cellular immunity [65, 137, 138]. Use of this background has been extensive across multiple groups and has allowed take rates in excess of 20% overall to be achieved on a routine basis (Table 3) ([69, 71, 54, 37, 139, 74, 140, 73, 141]; Gonzales-Suarez et al., unpublished; Bult et al. unpublished). The primary distinction of the NSG and NOG strains is that the function *Il2rg* in NSG is completely ablated. In NOG, the *Il2rg* mutation still produces a protein product that can bind to

cytokines, but there is no signaling activation. NSG mice are less susceptible to thymic lymphomas than NOD/SCID mice and have a longer lifespan, making them well-suited for engrafting slower-growing human tumors.

The NSG-recipient mouse has been shown to support greater engraftment of human hematopoietic stem cells (hu-CD34+ cells) than some other currently available strains [137, 142]. As a consequence NSG mice can be engrafted with functional human immune systems permitting the potential to study primary human tumors *in vivo* in the presence of a human immune system. However, NSG mice are also reported to develop lymphocytic neoplasms occasionally from human B- and T-cells co-transplanted with the human tumor fragments, which rapidly outgrow human epithelial tumor cells [143, 144]. Nonetheless, NSG mice are currently the most popular choice for developing breast cancer PDX, with the majority of the consortium groups using NSG exclusively or in combination with other strains.

An interesting modification of the NSG is the NRG strain, which replaces the SCID mutation with a *Rag1* mutation. These mice were used recently for the intraductal injection approach for PDX development [141]. Evaluation of NRG mice in experimental therapeutic studies involving DNA-damaging agents would be of considerable interest.

3.4.7 Comparisons of immunocompromised hosts with regard to breast cancer PDX modeling

With multiple host mouse models capable of generating PDX lines under a variety of conditions, using a broad range of tumor types, it is important to note that controlled head-to-head comparisons of single-tumor fragments transplanted into multiple immunocompromised host models under otherwise identical transplant conditions have not been conducted extensively. Thus, it is not currently possible to state definitively that one immunocompromised mouse model is superior to any other for generating breast PDXs. Similarly, comparison of take rates achieved between various groups is difficult due to considerable differences in tumor types used for transplantation (e.g., low-grade vs. high-grade primary cancers, primary tumors vs. metastatic tumors, before or after treatment) and patient cohort characteristics (e.g., ethnically diverse or not).

Limited side-by-side comparisons of engraftment efficiency have been performed, suggesting that the SCID/Bg or NOG/NSG backgrounds may provide superior engraftment of various types of human cells [122–125]. Indeed, when comparing two similar cohorts of breast cancer patients, primary tumors showed equivalent take rates in either the SCID/Bg or NSG backgrounds [54]. However, this study was not a head-to-head comparison using exactly the same patient sample into both backgrounds simultaneously. While the range of PDX models produced in these two backgrounds was similar,

it remains possible that each genetic background might favor the establishment of different types.

Some studies have now shown that positive engraftment of breast tumors into certain mouse strains correlates with high tumor grade or poor patient outcome [145, 71, 66, 99]. Whether the prognostic value of engraftment would extend to more immunocompromised hosts or whether retention of some cellular immunity in the host provides more information regarding tumor aggressiveness in the patient is still unknown.

3.5 What is the best transplantation site?

A variety of transplantation sites are available in the mouse, and several have been used in attempts to generate breast cancer PDX models. Tissue can be transplanted into the anterior compartment of the eye [146, 147], under the renal capsule [148–151], within the intrascapular fat pad [68], subcutaneously (ear, flank, etc.), or orthotopically within the mammary fat pad (either the intact (IFP) or epithelium-free “cleared” (CFP) fat pad) [152] or injected into the mammary ducts themselves [153, 154, 141].

In addition to these approaches for modeling primary tumors, “experimental metastasis” methods have been developed in which tumor cells are injected through the tail vein or the iliac artery or by intracardiac injection, allowing establishment of tumors at other organ sites including bone, brain, lung, and liver (e.g., [155–160] and references therein). A bone-to-bone xenograft experimental metastasis model has also been developed [161].

With respect to PDX model development, subcutaneous and orthotopic transplantation sites are generally used (Table 3), with orthotopic transplantation into the mammary fat pad considered preferable by some as it should more closely represent the human breast with respect to the microenvironment. However, the intraductal approach is also attractive with respect to the range of orthotopic options available. For example, in one study, cells from 16 tumors that successfully formed primary grafts after intraductal injection failed to engraft at 26 subcutaneous injection sites [141], but direct comparison of intraductal with sophisticated extraductal grafting techniques has not been performed.

3.6 Is it necessary to eliminate endogenous mouse epithelium from the mammary fat pad (i.e., “clear” the fat pad) for efficient orthotopic transplantation?

Elegant transplantation studies conducted nearly six decades ago [162, 152, 163] demonstrated that the adult mammary gland contains growth-quiescent epithelial regenerative stem cells that are distributed throughout the entire gland. These stem cells could be activated to self-renew upon transplantation of small duct fragments (~1000–2000 cells) into the epithelium-free mammary fat pad of recipient mice and were

capable of regenerating a morphologically normal and functional, ductal tree. However, these same fragments would not regenerate a ductal tree in the presence of preexisting ducts in the recipient animal. Thus, the presence of endogenous normal epithelium is inhibitory to transplanted epithelium, thereby necessitating removal of the endogenous epithelium (“clearing”) for the transplant to grow.

In contrast, neoplastic tissue transplanted into an intact fat pad will grow in the presence of endogenous normal epithelium. However, this ability does not necessarily mean that inhibitory signals do not exist, only that neoplastic tissue is capable of overcoming them, or is unresponsive to them, should they exist. Thus, while neoplastic tissue may be able to overgrow normal epithelium, removal of whatever inhibitory influences may exist might allow neoplastic tissue to grow even better. Conversely, it is formally possible that normal epithelium could promote growth of neoplastic tissue. These alternative hypotheses have not been tested in a controlled manner.

From inspection of Table 3, use of the intact mammary fat pad (IFP) does not appear to interfere with effective transplantation of primary tumor tissue. In fact, there are preliminary indications that the IFP may be superior to the CFP for transplantation of both fresh and previously cryopreserved tumor tissue (Table 3). That said, the continued presence of endogenous mouse epithelium may complicate some downstream molecular analyses (e.g., transcriptome arrays, reverse phase protein arrays (RPPAs), etc.), and so use of the IFP may not be desirable in all instances.

3.7 Is estradiol supplementation necessary? If so, what is the most effective way to deliver it?

With the possible exception of metastatic ER+ breast cancer, it has proven difficult to establish PDX models of primary, particularly lower-grade, ER+ luminal A tumors. Further, once established, ER expression can be lost over the course of passage from mouse to mouse (e.g., [68, 57, 164], Gomez-Miragaya et al. unpublished), though many current studies have not shown this behavior. These difficulties are offset, in part, by the fact that ER+ cancers account for approximately 75% of all breast cancers, thus offering more opportunities to make successful PDX models.

In attempts to increase the take rate of ER+ tumors, various forms of estradiol supplementation have been used, ranging from commercially prepared slow-release plastic pellets (e.g., Innovative Research) to hormone-loaded silastic tubes, or beeswax pellets. To date, progesterone supplementation, either alone or in combination with estradiol, has not been evaluated. Unfortunately, in addition to significant urinary tract complications associated with the use of estradiol-containing implants of all sorts, a major issue with this approach is that the implant becomes depleted of hormone over time, requiring

periodic replacement if hormone levels are to be maintained long-term. To address these issues, several groups have revisited use of an old method by testing the ability of estradiol-supplemented drinking water for its ability to support PDX growth with promising results (Table 3) (see [68, 165–168, 136]; Lewis et al., unpublished). Given the significant limitations of delivery using implants, estradiol-supplemented drinking water may be a superior method of delivery. However, it remains unclear whether rates of PDX establishment will be comparable to those using other delivery methods.

While most groups engaged in PDX generation use some form of estradiol supplementation for ER+ tumors, use of supplementation is not always used when transplanting ER- tumors (Table 3) [71, 114, 113]—the reasoning for not using supplementation being that ER- tumors should not require estradiol supplementation for their growth. However, comparison of stable take rates of primary cancers from statistically similar cohorts in the SCID/B_g host strain, with and without supplementation with slow-release estradiol pellets, demonstrated that supplementation increased the stable take rate almost 10-fold, from 2.6% without supplementation (only a single TNBC PDXs generated from 38 patients attempted) to 21% with supplementation (15 PDXs representing multiple IHC subtypes from 70 patients attempted) in this host background [54]. These data indicating a requirement for estradiol supplementation were consistent with previous studies showing that estradiol supplementation stimulates growth of breast cancer xenografts, including ER-negative xenografts [71, 169, 170, 69]. Subsequently, the stimulatory effect of estradiol supplementation on ER-negative tumor was investigated specifically and shown to be due, in part, to an effect on bone marrow-derived myeloid cells that promote angiogenesis and tumor growth that was dependent on ER α [131, 171].

Of potential significance, several groups have successfully used NSG mice without estradiol supplementation (Table 3), suggesting that unlike SCID/B_g, successful transplantation in NSG animals may not require supplementation. However, there may be significant differences in the patient cohorts and tumor characteristics from one group to another that may contribute to this apparent difference. That said, the intraductal approach appears to also one to grow ER+ cell lines and PDXs in the absence of endogenous hormones [154, 141].

It will be critical to address these potential host background differences going forward.

3.8 How can lower-grade tumors, ductal carcinoma *in situ*, and normal tissue be grown with higher efficiency?

While it has been comparatively easy to establish PDX models from metastatic sources such as ascites and pleural fluid, as well as from high-grade, more aggressive primary

tumors from the breast itself (Table 3), it has proven relatively difficult to establish PDXs from grade I/II tumors, ductal carcinoma *in situ* (DCIS), and normal breast epithelium [153, 75, 54, 66, 70, 114, 113].

One possible explanation for elevated take rates in high-grade primary tumors could be a higher frequency of tumor-initiating cells (TICs) (a.k.a. cancer stem cells) relative to lower-grade tumors [172, 173]. In one study, the frequency of cells expressing aldehyde dehydrogenase 1 (ALDH1), a marker associated with TIC properties in some, but not all, breast cancers, predicted the rate of engraftment as a PDX. The general hypothesis is that the higher the proportion of TIC, the higher the likelihood of successful transplant. This hypothesis makes intuitive sense from a statistical standpoint. However, if this were the entire explanation for differential take rates, transplantation of larger fragments of lower-grade tumors should address the problem of low take rates because more TIC would be present in the transplant. Given the widespread failure of several investigators to establish lower-grade tumors under a variety of conditions, this explanation, while plausible, is not likely to account entirely for the elevated take rate in high-grade cancers.

One other plausible explanation for differential take rates of low-grade (including DCIS) versus high-grade cancers is that immunocompromised mouse hosts do not express one or more factors that low-grade tumors require for growth in mice—factors that at least some higher-grade tumors do not require (analogous to a shift from hormone-dependent to hormone-independent tumor growth in some ER+ breast cancers).

In addition to this potential contribution of tumor evolution/progression, there are several known incompatibilities between human and mouse ligand/receptor pairs that may interfere with the ability to transplant human tumors into mice efficiently. For example, several mouse ligands (e.g., prolactin, hepatocyte growth factor (HGF), interleukin-6 (IL-6)) do not activate their human receptor counterparts [174–176]. Thus, tissue-appropriate expression of one or more of these human ligands either as transgenes or as knock-in constructs, in an immunocompromised mouse background, may be necessary to stimulate growth of lower-grade tumors.

One technical modification that may help with growth of lower-grade tumors is the use of Matrigel or similar basement membrane extract preparation in the transplantation process. Head-to-head comparisons of take rates with and without Matrigel using otherwise identical host mice and tissue sources have not been conducted. That said, a comparatively high take rate of 37% (10 of 27 patients) was achieved [69] using primary tumor fragments or metastatic cells (pleural fluid/ascites) coated in Matrigel and implanted into the intact fat pad of either estradiol-supplemented NOD/SCID or NSG mice. However, this reported take rate appears to have been attenuated with additional patient samples attempted

(Table 3). There also may be some indication that the use of Matrigel in the context of the IFP may help somewhat (Table 3). However, using previously cryopreserved tissue, the addition of Matrigel under otherwise identical transplant conditions does not appear to enhance the take rate (Table 3).

Another promising approach is the use of intraductal injection of cells. This method has been used to grow DCIS [153], ER+, and HER2+ breast cancer [154] and molecular apocrine cancers [141], with excellent recapitulation of the biology of the source tissue. The intraductal approach may allow human epithelium to interact with normal epithelium in much the same way that it would in a patient, thereby perhaps allowing growth whereas isolated fragments of human tissue may not grow in the absence of such interactions.

3.8.1 Progress toward development of mouse models of human breast premalignant lesions

For a comprehensive review of human DCIS models including *in vitro* models, please refer to Kaur et al. [177].

Efforts toward developing models of premalignant breast lesions go back to 1975 when Outzen and Custer reported transplantation of small fragments of human “cystic hyperplasias” into cleared mammary fat pads of nude mice and the lesions were maintained for 2–3 months [178]. The hyperplastic lesions proliferated in mice and recapitulated the histologic patterns of the original patient biopsy specimens [60]. Other investigators also reported that fragments from human breast atypical hyperplasias could be maintained for up to ~6 months in nude mice and, sometimes, the lesions would dedifferentiate, meaning that they potentially progressed by forming disorganized epithelial hyperplasias. Three transplantation sites were used, cleared mammary fat pad, subcutaneous, and intraperitoneal. The cleared mammary fat pad site was reported to result in the highest take rate (reviewed in [178]). Later, a study in 1997 reported transplantation of fragments from 25 cases of human ductal carcinoma *in situ* (DCIS) in the back of athymic nude mice [179]. Fragments were recovered 2–8 weeks after transplantation and maintained their DCIS components in 93% of transplants [179]. The purpose of this study was to analyze ER expression in the transplanted DCIS and to assess their response to estrogen supplementation. However, the authors reported no expansion in response to hormonal supplementation during the 8-week follow-up. In a more recent study, human DCIS tissue fragments were implanted subcutaneously in athymic nude mice in order to study the therapeutic efficacy of a farnesyltransferase inhibitor. The DCIS xenografts were maintained for up to 21 days and showed a take rate of about 66% [180]. Recently, Espina et al. demonstrated successful xenotransplantation of freshly procured DCIS organoids and *in vitro* propagated spheroids derived from patient DCIS biopsy or surgical specimens. This group reported that tumors

formed in mice at a rate of ~80% (21/27 cases transplanted) from either freshly procured organoids from DCIS of any grade or propagated DCIS organoids passaged *in vitro* for 2–12 months.

Toward the development of cell lines that would mimic the histologic and molecular features of premalignant breast lesions in xenografts, Miller and colleagues developed MCF10AT cell line models. Xenografted lesions derived from MCF10AT cells generated the full spectrum of human breast lesions including normal ducts, hyperplasia, atypical hyperplasia, carcinoma *in situ*, and invasive cancers [181, 182]. A clonal derivative of a tumorigenic variant of MCF10AT xenografts, MCF10DCIS.com, produced comedo DCIS when transplanted at early passages into cleared fat pads of immunodeficient mice. Subcutaneous injection of MCF10DCIS.com into nude mice resulted in rapidly growing lesions that were predominantly comedo (a more aggressive type of DCIS with central necrosis) [178]. When transplanted subcutaneously, the MCF10DCIS.com lesions appeared in about 3 weeks and were composed of luminal epithelial cells surrounded by both a myoepithelial cell layer and a basement membrane. Some areas of early lesions progressed to invasive cancers in about 5 to 6 weeks [183]. Another premalignant cell line model SUM225CWN was derived from a chest wall recurrence of a ductal carcinoma lesion [184]. Similar to those of MCF10DCIS.com, xenografts of the SUM225CWN cell line form DCIS-like lesions in NOD-SCID mice in as early as 2 weeks [185].

With the idea that human DCIS initiates inside the ducts, Behbod et al. utilized the intraductal transplantation technique [153]. This approach, referred to as mouse-intraductal (MIND), involves injection of epithelial cells derived from DCIS patient samples or cell lines directly into the immunocompromised mouse mammary ducts. This is the first model to capture the natural evolution of human DCIS in mice since, similar to humans, the cancer cells initially form *in situ* lesions inside the mammary ducts followed by invasion as they bypass the natural barriers of the ductal myoepithelial cell layer and the basement membrane. Initially, MCF10DCIS.com and SUM225CWN cells as well as one case of primary human DCIS were utilized. The DCIS-like lesions generated from the MCF10DCIS.com (DCIS.com) and SUM225CWN cell lines formed DCIS-like lesions as early as 2 weeks and slowly progressed to invasive lesions in 10–14 weeks [153]. Later, in 2011, this group reported reproducible growth and expansion of epithelial cells derived from patient DCIS biopsy and/or surgical samples as well as hyperplasias in NOD-SCID IL2r mice by the MIND method. The xenografted DCIS-like lesions and hyperplasias expressed similar biomarkers (ER, PR, and Her-2) as the original patient samples. The DCIS-like lesions generated by the primary DCIS cells in MIND models formed *in situ* lesions as early as 8 weeks, and a fraction of those slowly progressed to invasive lesions in 6–12 months.

following transplantation (Behbod, unpublished results). The take rate for primary DCIS MIND models is ~50%. A key difference of the MIND method is that pure epithelial cells are injected intraductally as opposed to previous methods that transplanted organoids or pieces of tissues in the cleared mammary fat pads of immunocompromised mice.

3.9 Can “humanization” of immunocompromised mice enhance take rate and improve translational relevance?

There are currently two different classes of “humanization” methods that are being explored for their ability to enhance PDX growth and perhaps provide a more biologically similar environment as in the tumor of origin.

3.9.1 “Humanization” of the mammary fat pad

A few groups have attempted to increase the efficiency of primary tissue transplantation by “humanizing” the mammary fat pad of mice with the introduction of an immortalized human fibroblast cell line into the mammary fat pad prior to xenograft transplantation [75, 87, 186]. Use of this methodology allowed organotypic growth of normal human mammary epithelium and appears to allow PDX establishment under some circumstances (Table 3). However, Zhang et al. [54] showed that provision of immortalized fibroblasts attenuated take rates considerably using statistically comparable cohorts in the SCID/Bg background reducing the rate from 21.4 to 3.4% under otherwise identical transplantation conditions. Thus, it remains unclear whether immortalized fibroblasts enhance PDX take rates.

Aside from immortalized fibroblasts, it is also possible that co-transplantation of mesenchymal stem cells may enhance PDX take rates. These cells were shown to enhance mammosphere formation *in vitro* [187, 188] and to stimulate growth and metastasis of established xenografts *in vivo* [71, 189, 190, 175]. However, this approach has not yet been tested extensively.

3.9.2 “Humanization” by reconstitution of the human immune system in immunocompromised mice

A disadvantage of any xenograft as a model for human cancer is growth of the tumors in immunocompromised mice, which is required in order to avoid rejection of the tumor by the host mouse immune system. Lack of normal immunity in tumor xenografts is an important caveat, given the well-established, multiple roles of the immune system in tumor initiation and growth, metastasis, and response to therapy. In most patients, tumors arise in the presence of a functional immune system, and the tumor evolves to evade immune rejection under selective pressure [191]. Once a tumor is detectable, it has already

avoided immune-mediated rejection, and thereafter, the immune system actually plays a paradoxical role in promoting tumor progression and metastasis [192–194]. For example, macrophages can assist with tumor cell intravasation to facilitate tumor dissemination [195]. Myeloid-derived suppressor cells can promote angiogenesis and suppress adaptive immune responses [196], and regulatory T-cells reinforce the immunosuppressive tumor microenvironment [197].

On the other hand, it has been clear for a number of years that the immune system can, in some cases, be successfully stimulated to eradicate tumors. Indeed, recent advances in immunotherapy using immune checkpoint inhibitors have led to exciting therapeutic results in certain cancers [198, 199]. The immune system is also instrumental in sustaining tumor regression upon oncogene inactivation [200], which is highly relevant when considering therapeutic effects. Therefore, incorporation of a functional or partially functional human immune system into xenograft models might more accurately model human breast cancer growth, metastasis, and response to therapy, particularly those known to involve an immune system function in their mechanism of action.

Generation of mice with reconstituted human immune systems was first accomplished in 1988 [201] and is now commonly used to study human blood cell development and diseases of the hematopoietic system [65]. The idea of using mice with “humanized” immune systems to study human cancer is not new, but until recently has been mostly focused on tumors of the hematopoietic lineage. Immune humanization in mice carrying human breast cancers is now feasible [202], with two key stipulations: (1) the mouse host has to be appropriate for growth and development of human tumor cells and human immune cells and (2) the immune system must not recognize the tumor as foreign and reject it (graft vs. tumor reaction). State-of-the-art strategies, as well as caveats, have been recently reviewed elsewhere [203–206].

In summary, it has become clear that the “tumor cell-centric” approach to breast cancer therapy may not be sufficient to eradicate the disease, and a dual approach targeting both the tumor and its specialized microenvironment might be more effective [207]. Many promising therapies for breast cancer rely on an effective anti-tumor immune response, and they need to be tested preclinically in animal models before entering trials. Examples include vaccines for breast cancer prevention or inhibiting outgrowth of occult metastasis [208], drugs to prevent macrophage recruitment into tumors where they promote metastasis [209], or new immune checkpoint inhibitor combinations [210]. In all cases, a better understanding of human tumor-immune interactions is needed. Future development of PDX models in mice with matched, functioning human immune systems may hold potential to advance breast cancer research and lead to new treatments.

3.10 What do we need to do to make PDX repositories truly clinically relevant? Toward development of data standards for “clinical” annotation of patient breast cancer samples and their associated PDX models

As a newly formed international consortium, we herein report details of a large collection of breast cancer PDX models that recapitulate patient biology to a high degree. However, the patient-related clinical data and PDX-related data collected by each individual group vary significantly, both in terms of content and terminology used. This review therefore provides an opportunity to begin a discussion about development of an international data standard that can be adopted by all PDX-generating groups to allow direct comparison of collections worldwide and provide the foundation for larger-scale international, multi-institutional collaborations. Ideally, data elements used should integrate well with existing data repositories and portals such as GEO, Oncomine, cBioPortal, etc., by having a shared conceptual understanding and similar terminology that allows direct mapping of data elements between PDX resources and data portals. Significant progress has been made in this area, including community discussions about standards for data format and content that will enhance data sharing and integration, which will be discussed in detail at a later date.

There are two broad classes of data that can be collected: patient-centric clinical data about the patient and tumor of origin and PDX-centric data about the characteristics of the xenografts themselves. Within these two broad classes, the amount of data that can potentially be collected is relatively large, while the ability to collect detailed data may be limited by personnel availability and financial support. In particular, with patient privacy laws in effect in various countries, care must be taken to firewall personally identifiable health information from other data that can be shared publicly without concern that privacy will be compromised. To accomplish this division requires dedicated “clinical” personnel with clearance to access and abstract clinical data and an independent group of dedicated “PDX” personnel with access to the abstracted clinical data, as well as PDX-based data elements and datasets. Finally, both of these efforts require significant involvement of bioinformatics and software development expertise for proper integration.

Clinical data can be collected with varying degrees of ease and can be divided into what might be considered a “minimal” or “essential” data set and a more expanded “ideal” data set at both the patient level and the sample level. At the patient level, it is essential that one knows the gender of the donor, the clinical event point that defines the sample type(s) that have been collected from the patient for use in PDX generation (e.g., benign/normal, primary tumor, second primary tumor, local regional recurrence, distant metastasis, unknown) and pathological stage (including nodal status, etc.).

In addition to these minimal data, there are several other data elements that greatly increase the utility of a renewable tissue resource for use in translational studies and drug evaluation. These include patient age at each clinical event point (e.g., biopsy, surgery at which time the sample used for generating a PDX was taken), parity history, race, ethnicity, family history of cancers, vital status and date of recording, and any sites of distant metastasis. These data would ideally also include treatments received by the patient, the timing of treatment relative to the time of tissue collection for the production of PDX models, and the treatment response of the tumor of origin (most easily evaluated in the neoadjuvant setting).

At the sample level, minimal data include pathological diagnosis of the sample taken, hormone receptor status (ER/PR) and percentage of positivity, HER2 status, and germline *BRCA1/2* mutation status of the tumor of origin for the resulting PDX lines to be maximally useful. Additional data of interest related to the sample used for PDX generation include Ki67 labeling index, status of commonly mutated genes like *TP53* and *PIK3CA*, pathologic stage and grade, molecular subtype (by multiple means), and treatment status at the time of collection. Short tandem repeat (STR) analysis is also essential, whenever possible, to ensure accurate patient/PDX relationships. Given the recent reports of selective outgrowth of Epstein-Barr virus-positive human lymphomas in attempts to generate solid tumor PDX models [143, 144], it is imperative that each PDX model be validated appropriately with cytokeratin and CD45 immunohistochemical staining.

Because the PDX itself is a renewable sample type, tissue is not limiting. Thus, the PDX can be characterized to a high degree without concern of exhausting the tissue, although maintaining low passage lines is a clear priority. Key PDX-based data types are essentially identical with sample-level data types collected clinically. Namely, it is critical to confirm the ER/PR/HER2 status in the PDX at different passages and to conduct STR analysis to establish the unique identity of the PDX line and to establish the direct patient/PDX relationship whenever possible, as well as for downstream quality control measures to ensure PDX identity and integrity over what are likely to be decades of passage in the future.

In addition to these minimal data, other data that are useful are PDX growth rates (doubling time), metastatic frequency (CTC, lung, liver, bone, lymph node, brain, etc.), and any number of “-omics” characterizations. The -omics characterizations possible to generate include, but are not limited to, genomic copy number alterations, mutation status in various genes (whole genome sequencing, exome sequencing, Sequenom, SNP analysis, etc.), methylome, gene expression at both the RNA level (RNAseq, array-based, QPCR, etc.) and the protein level (reverse phase protein array, mass spectrometry, Western blot, CyTOF), and metabolome (mass spectrometry-based), among other possibilities. All data can then be correlated to any subsequent behavior or response that a given

PDX, or set of PDX models, may have to experimental manipulation. Such correlations should be particularly important for evaluation of drug treatment responses (any number of agents, but specifically standard-of-care agents). To be particularly powerful, PDX-based -omics characterizations should be matched to the corresponding data derived from the tumor of origin whenever possible. However, given that patient samples are typically limited by size, this is not always possible.

4 Summary of strengths and limitations of PDX models for translational research and drug development

PDX models are potentially important tools for identifying mechanisms of *de novo* and acquired drug resistance, for identifying new biomarkers of breast cancer biology, for driving drug discovery, and for evaluation of new experimental therapeutics. Although superior to cell lines in recapitulating tumor heterogeneity, PDX models are also biased toward more aggressive tumors and the rate of engraftment can be an independent predictor of patient outcome. While the majority of PDX models represent TNBC, there has been substantial improvement in establishing HER2+ and ER+ luminal B tumors. As predicted, more differentiated ER+ tumors with a low Ki67 staining index are very difficult to engraft, and in cases where they do engraft, it is likely that selection for the most undifferentiated components of the tumor has occurred, resulting in a tumor quite different from that in the original patient. Thus, rates of engraftment are skewed toward the most undifferentiated subtypes of breast cancer and do not fully encompass inter-tumor heterogeneity.

The recent findings that the clonal dynamics of tumors are highly variable, ranging from minor changes on engraftment to extensive changes that accompany selection for a minor clone of originating cells, add a further degree of complexity [44]. Nevertheless, polyclonality is generally well-represented by breast xenografts, and they continue to serve as useful models provided their clonal repertoire is taken into consideration. Although metastatic lesions demonstrate improved take rates and growth, they cannot be used to study the process of metastasis from naïve tumors.

One of the major limitations of the PDX model is the deficiency of the mouse host immune system and selectively inappropriate microenvironmental influences. Severely immunodeficient hosts must necessarily be utilized, but these inevitably alter the growth kinetics of many PDX tumors and preclude evaluation of immune-modulatory therapies. The NSG mice are currently the most widely used strain, but these lack natural killer, B-, and T-lymphoid cells. Humanization of the immune system could in theory be achieved by mobilizing peripheral blood stem cells from the same patient, although this remains challenging on more than one front. Other

options include humanization of the mouse immune system by co-engraftment of human bone marrow cells [211].

In addition to immune system deficiency, PDX models lack human stromal components such as the different fibroblast populations, endothelial cells, and adipocytes, being replaced by their mouse counterparts which do not necessarily function identically. The growth factors and stromal requirements necessary for effective engraftment are poorly understood, and human fibroblasts are rapidly out-competed by mouse stromal cells following transplantation [71]. Genetic targeting of multiple human cytokine genes into their respective loci within mice [212] might improve tumor engraftment through the provision of crucial stroma-derived species-specific cytokines. In addition, one might expect that co-engraftment of mesenchymal stem cells or cancer-associated fibroblasts could enhance tumor growth and stabilize tumor heterogeneity. Despite these ideas, the key to fully efficient tumor engraftment is not still known.

PDX models have been shown to recapitulate the drug sensitivity responses observed in the tumors of patients, from which they were derived. However, the implementation of PDX mice as “Avatar models” to guide clinical decisions will require significant advances in this area, including rigorous comparison of pharmacokinetics and pharmacodynamics for various agents that will allow “preclinical trials” in PDX-bearing mice to be as comparable as possible to clinical trials as conducted with patients. Aside from the limitations owing to lack of a human immune or stromal system, one of the limiting issues is the time-scale required to establish PDX models for drug testing. We also need to collect data about adverse drug reactions in different mouse strains. Furthermore, not all patient tumors engraft, adding a level of unpredictability. In order for PDXs to be relevant to the individual patient, the engraftment rate and time required for engraftment need to be dramatically optimized and standardized, without compromising the biological properties of the original patient tumor.

At this stage in their credentialing, PDX models best serve as basic and translational research tools, where they can have considerable impact. One of the best examples has been seen in the case of colorectal cancer, where PDX studies showed that tumors with a mutated KRAS gene were not responsive to cetuximab [213], closely mimicking that seen in large clinical trials. These results provide compelling support for PDX models as predictors of clinical response, and their further implementation could circumvent long-term, costly clinical trials in the future [214].

5 Focus for the future

At some point, perhaps even very soon, the more aggressive forms of breast cancer will be represented well enough in the

PDX community that it is no longer necessary to expend additional resources and effort to develop new PDX lines to represent them. Rather, it may be more fruitful to redirect resources to generate PDX lines for underrepresented tumor types or patient populations. These underrepresented tumor types and populations may offer insights into breast disease that would not be found otherwise and would likely facilitate development of personalized medicine strategies.

It has already been mentioned that ER+ luminal A and HER2+ tumors, grade I/II tumors, DCIS, and hyperplasias are significantly underrepresented in the PDX collections reported herein, but the increased use of the intraductal approach may remedy this situation. Similarly, there are very few claudin-low or metaplastic tumors present in the current collection; these are tumors with very poor prognosis and limited treatment options. As such, focused effort to establish PDX models representing these underrepresented tumor types may be preferable to the broader efforts currently underway.

In addition to these more familiar tumor types, there are other classes of tumors that are also underrepresented, on which focused attention might be warranted. For example, while some models do exist, tumors from Hispanic, African American, Asian, and Native American and other indigenous peoples are still underrepresented. Similarly, while males do develop breast cancers, there are presently no male breast cancers represented in the PDX collections presented herein. Finally, if the proper infrastructure were developed, we have the opportunity to try to generate PDX models from areas of the world in which health care is limited, and PDX generation efforts are entirely lacking. Together, these rare and underrepresented PDX models may provide unexpected insights that cannot be foreseen.

6 Conclusions

1. PDX models representing clinically relevant subtypes of breast cancer are available as phenotypically stable, renewable tissue lines.
2. Breast cancer PDXs recapitulate many key aspects of the biology of the tumor of origin and therefore may serve as excellent models for translational research.

Key unanswered questions

1. To what extent can PDX models be used as patient “avatars” in preclinical evaluation of experimental therapeutics?
2. Can PDX-based “-omics” studies be used to develop predictive signatures and to identify key resistance mechanisms?

3. Under what circumstances does the lack of an intact immune system influence the usefulness of PDX models, and can this be overcome?

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