CANCER MODELS

A new sophistication for breast cancer PDXs

New research shows that comprehensively characterized patient-derived xenografts (PDXs) of breast cancer can be adapted to high-throughput drug screening and can be used as personalized patient avatars to inform clinical decision-making. This work substantially enhances the repertoire and sophistication of PDXs for research into breast cancer.

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The development of robust models for human disease states, complete with deep characterization and clinical follow-up, is instrumental to the acceleration of translational research, particularly for diseases of current unmet clinical need. To that end, patient-derived xenografts (PDXs) have long been considered the gold standard for testing novel cancer therapies, among other preclinical research applications. PDXs are established by the implantation of tumor tissue collected from patients into immunocompromised mouse hosts and have been shown over the years to recapitulate many aspects of the tumor-intrinsic biology, including intra-tumoral heterogeneity; genetic, genomic and epigenetic states; tumor growth and metastasis; and, crucially, drug responses[1–4] (Fig. 1). With the growing appreciation that PDXs more closely model human tumor biology than do conventional two-dimensional cell lines, the research community is amassing PDX biobanks across many cancer types, including breast cancer. Writing in *Nature Cancer*, Guillen et al. now report the development and characterization of an extensive biobank of breast cancer PDX models that, notably, contains a considerable representation of deadly, high-risk, metastatic and treatment-resistant models[5]. Furthermore, they have devised a straightforward method for converting the PDX biobank into patient-derived xenograft organoids (PDxOs), to allow high-throughput and cost-effective in vitro screening. Finally, with a case study of a patient with metastatic breast cancer, the authors offer a tantalizing glimpse into a potential future for personalized medicine in breast cancer using PDXs as personalized patient avatars.

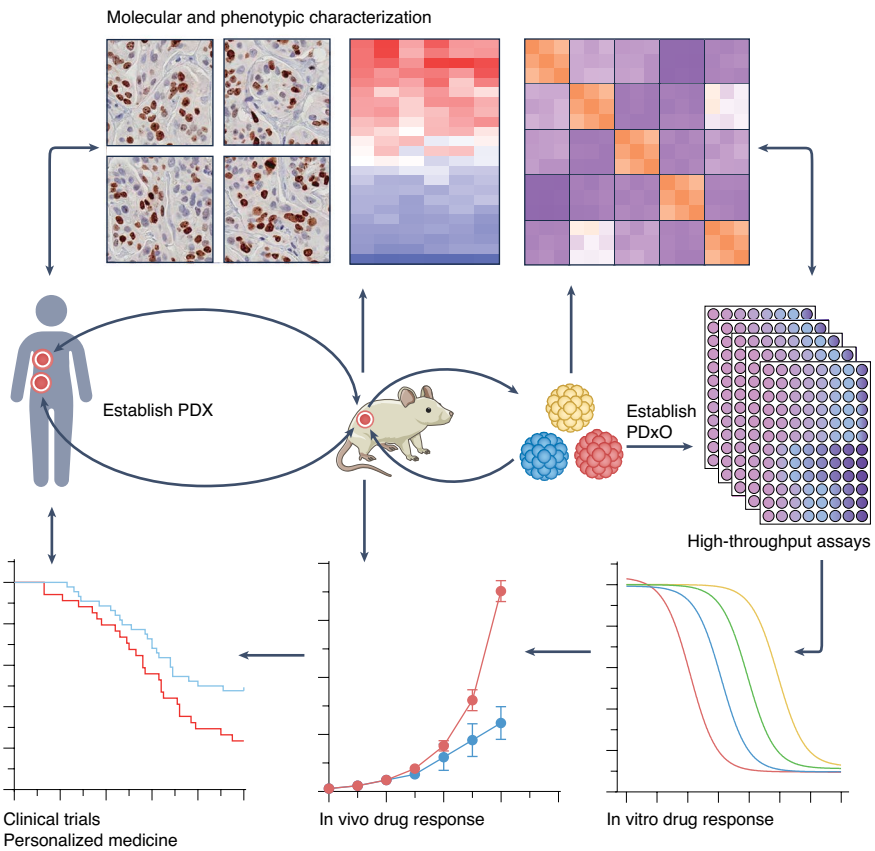

Fig. 1 | The establishment and applications of PDX and PDxO models. The establishment of PDX and PDxO models from donated patient tissue, when accompanied by detailed molecular characterization and high-throughput drug response data, feed back vital insights that allow the development of novel clinical interventions and personalized medicine strategies, continuing the fundamental bench-to-bedside-to-bench cycle of modern biomedical research.

Guillen et al. employed a comprehensive characterization of over 40 new breast cancer PDX models that included genomic sequencing across a panel of oncogenic drivers, transcriptional analysis for molecular subtyping using the PAM50 genes (a 50-gene signature that classifies breast cancers into five molecular intrinsic subtypes), profiling of copy-number variation, and histological staining for key diagnostic markers of breast cancer[5]. In each case, a high concordance was found between the newly developed PDX models and the originating patient tumors. The efforts of these authors address a pivotal concern in the field, as the utility of PDX biobanks as a research tool relies on rigorous and comprehensive characterization to establish the degree of concordance with the target patient population[6]. In fact, a recent meta-analysis of publications reporting derivation of new PDX models found that over 50% of reports in the setting of breast cancer were of high concern due to insufficient validation[7]. A minimal dataset

for reporting newly derived PDX models has been proposed[6] that suggests the deposition of detailed information across four key areas: clinical data of the patient (e.g., gender, age and treatment history); clinical data of the tumor (e.g., tissue of origin, tissue histology and whether the tumor came from a primary or metastatic site); methodological details (e.g., mouse strain, engraftment site and humanized immune system); and quality-assurance metrics (e.g., tumor characterization and animal health metrics). Additional data related to treatment responses, molecular '-omics' data, growth kinetics and model availability are desirable when available. In their current publication, Guillen et al. have fully embraced this ideal to deliver a comprehensive characterization of their new breast PDX library that covers not only the key data but also the vital '-omics' and treatment-response data that allow these models to be immediately utilized as a platform for biomarker discovery, drug testing and studies of fundamental tumor biology[5]. As tumor subtypes continue to be demarcated ever more finely, the detailed molecular phenotyping of models demonstrated here will become increasingly important, particularly in the treatment-resistant setting, in which novel tumor biologies evolve in response to treatment.

The 'take rate' (percentage of models implanted that can be stably grown in the mouse hosts) is relatively low in breast cancer; Guillen et al. reported a 29% success rate[5], which is consistent with the field and reflects a well-known selection bias in PDXs for tumors with a higher proliferative index. Successful engraftment rates differ between breast cancer subtypes. In particular, estrogen receptor–positive (ER+) disease, despite being by far the most common breast cancer subtype, is comparatively under-represented in PDX libraries due to its lower take rate and slower growth than that of less common but more aggressive tumor types[8]. Nevertheless, Guillen et al. have established eight ER+ PDXs[5] that have been additionally characterized for critical endocrine-resistance drivers such as mutations in *ESR1* (which encodes an estrogen receptor)[9] and for sensitivity to estrogen. Notably, clinical annotation established that a number of these ER+ models were derived from metastatic sites from patients whose cancer had progressed on endocrine therapy. Metastatic and treatment-refractory settings are in urgent need of new treatment strategies; thus, the biobank from Guillen et al[5]. provides a much-needed increase in coverage of these under-represented experimental models.

With the PDX catalog in hand, Guillen et al. also took steps to address the issue of throughput in translational research models[5]. Although PDXs as a model system outperform cell lines in their ability to recapitulate human disease states, as a trade-off, PDX models carry the major limitations of high cost and low throughput associated with growing tumors in mouse hosts. As there is now a clearer appreciation for intra-patient and inter-patient tumor heterogeneity at the molecular level, it is critical that novel therapeutic strategies are trialed across a range of representative models. This will allow broader insight into the spectrum of treatment sensitivities and perhaps the discovery of new biology that may underlie unexpected variations in response. Several methods have been developed to enable this where PDX models are impractical, including pared-down experimental designs, two-dimensional patient-derived cell cultures, and three-dimensional PDxOs[8]. PDxOs can be used for robust experiments with plenty of technical and biological replicates with improved stability relative to that of two-dimensional cultures, and thus allow longitudinal and replicative studies[4].

Guillen et al. transferred their PDX models to three-dimensional cultures with an impressive 85% success rate[5]. Over long-term culture, the PDxO models maintained consistent gene-expression phenotypes, and most could be transplanted back into mice with no change to growth kinetics compared with that of the original donor PDX. Analyzing a subset of their matched patient–PDX–PDxO models, the authors found excellent correlations between driver mutations, copy-number variations, the overall transcriptome, and epigenetic methylation states. Perhaps most notably, given the major utility of PDxOs for drug screening, the PDxOs exhibited the expected drug response based on their molecular profile (e.g., ER+ models responded well to ER-directed therapies but were less sensitive to chemotherapy) in a high-throughput screen against 45 therapeutic compounds that, for a subset, was mirrored in the parental PDX. These findings provide confidence that PDxOs are a robust and reproducible alternative to PDXs for large-scale drug screening. For example, the PDxO screen predicted that half the models of triple-negative breast cancer would be sensitive to the apoptosis inducer birinapant, a drug that has been shown to sensitize this cancer to chemotherapy. This prediction was fully recapitulated when the parental PDX models were exposed to this drug in vivo.

Although PDxOs bridge the gap between traditional cell line–based in vitro studies and in vivo PDX studies, a shared limitation is the inability of in vitro culture conditions or immunocompromised mouse hosts to model the human tumor microenvironment. This may result in fundamental differences in tumor biology and the way in which models respond to therapies that target ligand-dependent pathways that involve signaling from the microenvironment. Indeed, Guillen et al. reported significant changes in the expression of ER and in ER-dependent transcription in their PDxOs after a long time in culture[5]. Furthermore, the lack of a fully functional immune system in either the mouse host or in the PDxO setting hinders the evaluation of immunotherapy strategies that are increasingly the subject of preclinical and clinical investigation. Without further interventions, such as the introduction of a humanized immune system into the mouse host[10], PDXs and PDxOs cannot be used in studies of therapies directed against the immune system and may not even give an accurate portrayal of response to therapies that act partially through involvement of the immune system, such as antibody-dependent cellular cytotoxicity in the context of monoclonal antibodies to the growth-factor receptor HER2.

A major aspiration for translational research and precision medicine is that patient-derived models may be deployed as a key adjunct to clinical decision-making, acting as a personalized avatar for the patient for rapid molecular analysis and testing of potential therapies[11]. Barriers to this ambition in the setting of breast cancer include relatively low take rates, which means many patients, particularly those with ER+ tumors, will not have tumors suitable for PDX development; and slow growth rates, which means that insights cannot be delivered in a cost-effective manner within clinically relevant time frames. Clarification of the determining factors for PDX engraftment, through a comparison of the primary samples that succeeded or failed to engraft, would go some way to overcoming the first of these barriers, which remains to be done in a systematic fashion. PDxOs seem to be a very promising prospect for addressing the second barrier. As proof of concept for this, Guillen et al. were able to recommend an alternative therapeutic strategy for a patient who had donated her tumor for PDX generation and whose cancer had subsequently progressed on standard-of-care therapy[5]. With insights gained from the PDxO screen, the patient received eribulin, which resulted in a treatment response and a significantly longer progression-free survival than that after prior therapies. This case study underlines the importance of ongoing efforts to develop

new methods and strategies that can translate this indispensable research tool into an equally important tool in the clinician's bag.

PDX models are a vitally important component of modern cancer research, but the utility of any model system is reliant on a confident understanding of its strengths and limits. Models generated from patients that cover the spectrum of clinical progression and drug resistance are of high translational relevance, as this is the setting in which the majority of drugs will enter clinical trials. With a balanced focus on characterizing the cancer biology and drug response, the PDX library published by Guillen et al[5]. represents a considerable expansion of available patient-derived models that will provide researchers with important tools in their urgent battle against treatment resistance and metastatic progression. ❐

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Competing interests

The authors declare no competing interests.