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Review

# Estrogen receptor positive breast cancer patient–derived xenograft models in translational research

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**Abstract**

Estrogen receptor (ER) positive patient–derived xenograft (PDX) models of breast cancer are important translational tools in our pursuit for a better understanding of treatment resistance and for the preclinical evaluation of novel therapies. PDX modelling of ER+ breast cancer is traditionally associated with caveats such as low engraftment rates and absence of an immune microenvironment, leading to a paucity of ER+ models and an inability to assess immune-related effects. Furthermore, with the increased demand for modelling of therapy-resistant metastatic ER+ disease, our approach to propagating these models needs to evolve to ensure accurate recapitulation of the clinical features observed in patients with treatment-resistant breast cancer. In this review, we discuss recent major advancements in this field and the increasing utility of these models for high throughput screening of novel therapeutics.

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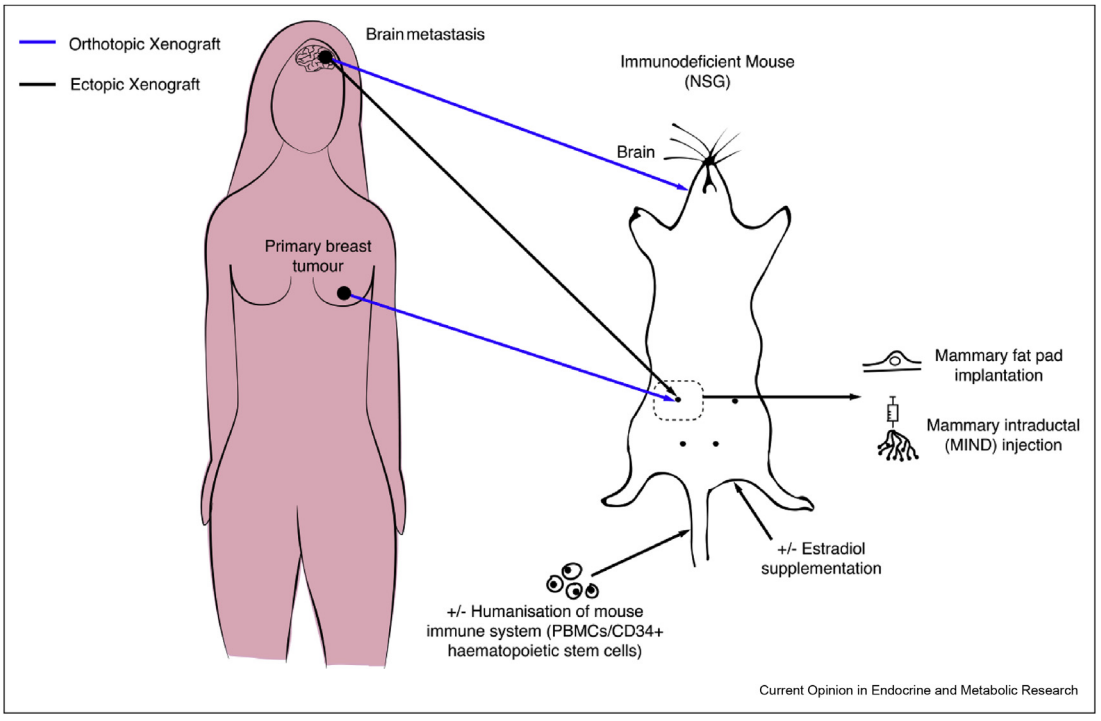
## Introduction

Patient-derived xenografts (PDX) are now widely used as an important preclinical tool in oncology to further our understanding of tumour biology across a range of malignancies. They are established through the implantation of patient tumour tissue into an immunodeficient murine host and have been shown to preserve the proteomic, transcriptomic and genomic features of the donor tissue and to recapitulate patients' response to therapy [1,2]. As PDX use becomes increasingly widespread, guidelines have been established for a minimum data set required for characterisation of PDX models [3,4]. These guidelines recommend the inclusion of essential and desirable information, which will foster consistency in the reporting of PDX models. Additionally, they allow for clarity of interpreting the significance of results achieved with these models. Essential information includes the details about source of the tissue, such as gender, age and diagnosis, clinical information such as whether it is a primary or metastatic sample and desirable information pertaining to the patient treatment history and the response of the PDX to standard-of-care therapies.

Breast cancer PDX models are routinely propagated in the mouse mammary fat pad and have enhanced our understanding of breast cancer biology pertaining to intra-tumoral heterogeneity and changes in clonality following therapy [5–7], as well as in the preclinical evaluation of novel targeted therapies [8–10]. The success of developing breast cancer PDX models in the mammary fat pad is subtype-dependent and favours tumours with basal differentiation and a higher proliferation rate such as triple negative breast cancer (TNBC) [11,12]. Hence, whilst estrogen receptor positive (ER+) breast cancer is the most common breast cancer subtype, accounting for over 70% of cases, there is a relative scarcity of these PDX models available for research. By improving the relatively low take-rate, optimising the approach to propagate ER+ breast cancer PDXs, including therapy-resistant disease, and establishing these in an immune competent environment, the clinical utility of these valuable resources could be further enhanced. This review focusses on recent technical developments (Figure 1) to improve the establishment and utility of ER+ PDX models for translation research.

**Figure 1**




Summary of technical developments in the establishment of ER+ PDX tumours. Technical improvements include orthotopic implantation of metastatic tissues, no requirement for estradiol supplementation, mammary intraductal injection of epithelial cells and humanization of the immune system of the murine NSG hosts via transplantation with peripheral blood mononuclear cells (PBMCs) or CD34+ haematopoietic stem cells.

## Mammary intraductal (MIND) tumour implantation

Human breast cancer xenografts have traditionally been established by injecting human breast cancer cells embedded in Matrigel into the murine mammary fat pad. Engraftment can also be achieved via injection of breast cancer cells into the mammary milk ducts, which may provide a micro-environment more conducive to preserving the biology and natural history of ER+ models [13,14]. A landmark study demonstrated that injection of ER+ MCF7 cells into the mammary fat pad—induced basal differentiation of the cancer cells as a result of increased TGFβ/SLUG signalling, and resulted in highly proliferative ER+ xenografts with lower expression of steroid hormone receptors such as ER and the androgen receptor (AR) [15]. This shift towards a more proliferative phenotype in ER+ tumours is supported by reports that most ER+ PDX tumours are classed as the more proliferative luminal B subtype at the transcriptomic level, even when the matching patient tissues implanted were of the less proliferative luminal A subtype [16]. In contrast, SLUG signalling was suppressed when these cells were injected into the mammary milk ducts. This resulted in the development of ER+ MCF7 mammary intraductal (MIND) tumours, which maintained a luminal A phenotype characterised by higher expression of steroid receptors and a lower proliferation index [15]. Furthermore, these MIND tumours responded to endocrine therapy and, unlike most mammary fat pad implanted tumours, did not require exogenous estradiol supplementation to grow, hence avoiding the issue of estradiol-induced toxicity [17].

The applicability of this intraductal approach was evaluated using patient-derived ER+ breast cancer samples and the resultant MIND ER+ PDX models were found to harbour similar histopathological and therapeutic response characteristics to the patient from which it was derived from [18,19]. A challenge with the use of MIND, particularly for experiments in which tumour growth kinetics are an endpoint, is the difficulty in obtaining accurate tumour measurements. MIND tumours do not grow in the typical spherical fashion of traditional fat pad xenografts, which are amenable to manual measurement using callipers. Further manipulation of the models to transduce a luminescent marker prior to implantation is required to infer MIND tumour volume changes using a bioluminescent *in vivo* imaging system [15,19].

## Establishment of therapy-resistant ER+ PDX models

Most PDX models of therapy-resistant ER+ breast cancer are established from patients who have progressed on therapy, often from biopsies from metastatic sites or from residual primary tumour following neoadjuvant systemic therapy, or following chronic exposure to therapy in the animals. In the context of ER+ breast cancer, mutations in *ESR1* (e.g. *Y*537S and *D538G*) have emerged as one of the most prevalent mechanisms of endocrine resistance, and can be found in up to 40% of patients with ER+ breast cancer who had progressed on aromatase inhibitor therapy [20–22]. ER signalling is altered in the endocrine-resistant context including those harbouring *ESR1 mutations* [23,24]. Due to the diversity of mechanisms of endocrine resistance, it is critical that the underlying drivers are identified in each model through in-depth genomic, transcriptomic and proteomic characterisation of the tumour, and these mechanisms of resistance can be used as biomarkers of response to novel targeted therapies [25]. Importantly, one cannot assume that PDX models will always recapitulate the therapy response of the patient in which it was derived from, and it is important to validate this *in vivo* as part of the characterization of the PDX model. The patient treatment history and PDX model's response to therapy should be included in the minimal information provided when using ER+ PDX models [4].

An important consideration in the establishment and propagation of endocrine-resistant ER+ PDX models is whether estradiol supplementation should be routinely used. Estradiol has been shown to have a paradoxical growth suppressive effect on some endocrine-resistant models [16,26,27]. Furthermore, *ESR1* mutations result in the constitutive activation of ER in a ligand-independent manner consistent with the ability of ER+ tumour cells harbouring these mutations to circumvent oestradiol-deprived conditions [28]. Estradiol supplementation in this context may potentially reactivate classical ER signalling-dependent proliferation and render these models biologically more similar to treatment-naive ER+ breast cancers [17,24]. Thus, it is critical to evaluate these models in the presence and absence of estradiol to more accurately determine the growth conditions of the tumour at baseline prior to using these models to evaluate the effects of novel therapies [8,10].

## Orthotopic versus ectopic implantation of metastatic tissues

Another important consideration in the establishment of PDX models is the origin of the tissue from which the tumour was derived. This is particularly relevant in the study of metastatic disease which is the main cause of breast cancer–related mortality. In contrast to PDX models from primary breast cancer which are implanted orthotopically into the mammary fat pad, metastatic tissues are routinely implanted ectopically into the fat pad or subcutaneously. In one study, transcriptomic analyses revealed that organ-matched orthotopic PDX tumours were more similar to the donor tissue than mammary fat pad implanted metastatic tumours [29]. In addition, there were differences noted between the chemotherapy sensitivity in mammary gland implanted PDX tumours compared to the matching orthotopic brain implanted tumours. These observations support the hypothesis that the metastatic niche is important for the establishment, growth and therapeutic response of disseminated cancer cells [30]. These are important considerations in PDX models derived from metastatic sites when interpreting inferences regarding their biology and therapeutic response. A major limitation of this technique is the technical complexity in engrafting in vital organs such as the brain, liver, lung and bone, and the limited ability to measure tumour responses in these internal organs in the mouse without advanced imaging techniques. Thus, this approach is still limited in its utility at this current time.

## Utility of PDX models for drug screening

High husbandry costs, particularly in the context of ER+ breast cancer where many models grow relatively slowly and mice must be maintained for long periods, have limited the number of biological replicates used in experiments designed for preclinical evaluation of therapies. The number of models tested must be balanced against the number of technical replicates (i.e. mice) required per treatment arm for statistical robustness, and the number of treatment arms to be studied. There have been efforts made to reconsider this approach in order to increase the scalability and decrease the associated costs of including a wider biological diversity in therapeutic studies. The $1 \times 1 \times 1$ experimental design is an approach whereby drug screening of therapeutics is carried out in a panel of well-characterized PDX models with each model implanted into a single mouse. This approach sacrifices technical replicates for biological replicates and derives statistical power from the population response [31]. In the largest study of this kind, response to therapeutics of 29–45 different PDX models of several tumour types were studied. The authors identified novel synergistic drug combinations as well as novel gene mutations which can confer resistance to these therapies [31]. Given that this screening method recapitulates clinical scenarios i.e. each PDX model represents a single data point as each patient in a trial represents a single data point, it is proposed that this high throughput approach of therapeutic screening would improve the ability of preclinical studies to predict clinical response in patients. However, this approach relies upon economies of scale and requires a large

library of well-characterized PDX models. As such, apart from well-resourced pharmaceutical companies, the high cost of establishing such an infrastructure is likely to render this approach out of reach for most academic investigators.

A more economical approach may be to perform therapeutic screening on organoids [32] or short-term patient-derived tumour cell cultures derived from PDX tumours [1], where PDXs serve as intermediate hosts to expand limited patient-derived tissue. These *ex vivo* resources can be set up after digestion of the PDX tumours into either organoids [32] or single cells [1] using commonly available reagents. Importantly, the response of the organoids or explants to therapeutics has been shown to mimic the response of tumours *in vivo* in both these studies, representing a cost-effective strategy to select candidate drugs from a larger library for subsequent *in vivo* evaluation.

## Overcoming limitations of immunodeficient murine hosts

Immunodeficient murine hosts such as the NOD/SCID and NOD/SCID/Gamma (NSG) mice have been used for the development of breast PDX models [33–35]. NOD/SCID and NSG mice are deficient in immune functions pertaining to macrophages, dendritic cells, T cells and B cells, with NSG mice further impaired in natural killer cell functions. A major caveat of using immunodeficient murine models is the inability to assess the effect of therapy on the immune system and the effects of immunotherapy, which has shown promise in the treatment of TNBC [36]. The lack of immune cells in these murine models can now be overcome by using human immune progenitor cells to reconstitute an immune system in the mouse leading to the establishment of humanised mouse hosts. This can be achieved by transplantation, via tail vein injection, of peripheral blood mononuclear cells (PBMCs) obtained from patient donors [37] or CD34+ haematopoietic progenitor cells isolated from cord blood, human liver or thymus [38–41]. Immune reconstitution with PBMCs results in the reconstitution of T lymphocytes, B lymphocytes, monocytes and natural killer cells, while transplantation with CD34+ haematopoietic stem cells reconstitutes T and B lymphocytes. A major limitation with PBMC transplantation is the acute onset (4–6 weeks) of graft-versus-host disease (GVHD), whereby the human lymphocytes target host organs due to recognition of the murine major histocompatibility complexes (MHC) I and II. This terminal development limits the therapeutic window for studies with these models [42], and a challenge in ER+ PDXs which typically take a longer time to grow. More recently, an NSG mouse model deficient in MHC Class I and II expression has been developed to overcome GVHD, and may become the mouse recipient model of choice for PBMC reconstitution [43]. In contrast, GVHD is rarely observed up to 6 months post engraftment in CD34+ haematopoietic stem cell (HSC) transplantation [44], and has been used to assess therapies that act through immune mechanisms such as inhibitors of PD-1 (Pembrolizumab), PD-L1 (Nivolumab) and CTLA-4 (ipilimumab) in TNBC PDX and cell line xenograft models [38–40].

The interaction between immune and cancer cells is an emerging area of investigation in ER+ breast cancer [45,46]. The evaluation of immunotherapy in ER+ breast cancer is currently lagging behind other types of cancer and its efficacy is still not clear [47]. CDK4/6 inhibitors, which are the new standard-of-care therapies in ER+ metastatic breast cancer [48,49], have been reported to induce T cell activation in murine syngeneic models, highlighting the importance of studying immune responses with these therapies [50,51]. A humanised immune system is a promising development in the field of PDX modelling, as it provides an opportunity to evaluate immunotherapy preclinically in breast cancer PDX models. This strategy has yet to be deployed on ER+ PDX models, and with their inherent slower growth rates will necessitate feasibility studies to assess if the growth of ER+ tumours in this humanised environment allows for therapeutic interventions before the onset of GVHD.

## Conclusion

PDX models are currently considered the gold-standard of preclinical modelling. Early work demonstrating how closely PDX models recapitulated clinical scenarios when compared to *in vitro* or cell line xenograft models secured their place as the final gateway to translation of preclinical findings. However, as the use of PDX models has become increasingly widespread, our understanding of cancer and the sophistication of the questions that we use PDX models to answer has similarly increased, particularly in regard to the contribution and targeting of the microenvironment and the development of resistance to standard-of-care therapies. It has become increasingly apparent that our approach to modelling needs to evolve with our understanding of tumour biology, including the tumour environmental niche. This has brought many challenges. Some are common to all PDXs, such as the contribution of the immune system, whilst others are specific to particular tumour types. In terms of modelling ER+ breast cancer, key challenges include improving the take-rate of ER+ models; the appositeness of fat pad implantation and the use of exogenous estradiol in the establishment of endocrine-resistant PDX models. In addition, the reconciliation of relatively low growth rates with the cost of husbandry for large experiments of sufficient biological diversity to accurately inform clinical trials remains a challenge.

Continuing work is addressing each of these challenges and new methods and techniques continue to further improve the recapitulation of clinical scenarios across a range of increasingly complex metrics encompassing tumour growth; transcriptional, proteomic and epigenetic responses; and the contribution of the tumour microenvironment. The incorporation of high throughput *ex vivo* and *in vivo* drug screen methods will allow us to capture a wider range of ER+ models with different therapy resistance mechanisms and greatly improve the repertoire of therapies that can be evaluated.

The role of PDX models in preclinical oncology research continues to develop. Ongoing efforts to further finesse the clinical accuracy of the models and the development of minimal reporting standards (which will in turn need to evolve as modelling becomes more sophisticated) will only increase the utility of these models and cement their place as critical pre-requisites for drug development.

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## Conflict of interest statement

Nothing declared.

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