REVIEW

The challenges of modeling hormone receptor-positive breast cancer in mice

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Abstract

Estrogen receptor-positive (ER+) tumors account for 70–80% of all breast cancer (BC) cases and are characterized by estrogen dependency for their growth. Endocrine therapies using estrogen receptor antagonists or aromatase inhibitors represent a key component of the standard of care for these tumors. The occurrence of de novo or acquired resistance to estrogen withdrawal represents an important clinical problem, impacting on patient survival. In addition, despite an initially favorable outcome, a part of ER+ BC patients present with disease recurrence locally or at distant sites years or even decades after apparent remission. *In vivo* models that closely mimic human disease are urgently needed to study the biology of these tumors, investigate the molecular mechanisms underlying endocrine resistance and identify patients at risk of recurrence. Despite the similarities in the overall hormonal regulation of mammary gland development between mice and humans, the majority of the mammary carcinomas occurring in genetically engineered mouse models (GEMMs) are ER negative and most xenograft models are based on few ER+ cancer cell lines. We recently showed that the microenvironment is critical for ER+ cancer cells and discuss in this review the potential of intraductal xenograft model for basic and preclinical research.

Key Words

- ▶ estrogen receptor
- ▶ metastasis
- ▶ breast cancer
- ▶ preclinical model
- ▶ endocrine therapy

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Introduction

Breast cancer is the most frequently diagnosed non-skin cancer in women and remains the leading cause of cancer death in women (Siegel *et al.* 2016). Breast cancer (BC) is a heterogeneous disease comprising various subtypes with distinct histopathological and clinical characteristics. Tumors are traditionally classified based on the presence or absence of estrogen receptor (ER), progesterone receptor (PR) and the human epidermal growth factor receptor 2 (HER2) as assessed by immunohistochemistry (IHC) or/and *in situ* hybridization (ISH). Tumors lacking expression of all three receptors are called triple negative

(TN). Hormone receptor-positive tumors account for about 70–80% of all invasive BC cases (Bentzon *et al.* 2008, Yanagawa *et al.* 2012) and largely overlap with the two luminal molecular subtypes (Perou *et al.* 2000). Luminal A and B tumors are distinguished by a cell proliferation signature, which is reflected in the IHC by a higher Ki67 index (with a cutoff around 20%) in luminal B BC (Schnitt 2010). Most ER+ BCs are of no special type (NST), the most frequent special histological subtype is the invasive lobular carcinoma (ILC), which accounts for 10–15% of all BC cases (Cristofanilli *et al.* 2005a).

The incidence and outcome of the various subtypes vary with age, race and menopausal status (Carey *et al.* 2006), and the different clinical and molecular features allow assessment of prognosis and assignment of the most appropriate treatment for patients on an individual basis (Schnitt 2010). ER expression is at least initially, associated with a more favorable prognosis and is predictive for responsiveness to endocrine therapies (Bentzon *et al.* 2008). ER+ tumors depend on estrogen for growth, and the development of endocrine therapies, which target ER signaling, has been one of the greatest clinical advances of the past 50 years. Indeed, it has served as a paradigm for targeted therapies in oncology (Sledge *et al.* 2014). However, about 20% of the patients do not respond to first-line endocrine therapies and up to 50% acquire resistance under treatment through different mechanisms (Murphy & Dickler 2016). In addition, ER+ cancers are susceptible to relapse at distant sites, predominantly in bone, after years or even decades of apparent remission. This phenomenon has been attributed to the presence of ER+ tumor cells that may remain dormant in different organs and become reactivated, possibly as a result of alterations in immunosurveillance and microenvironmental signals (Zhang *et al.* 2013b). Thus, despite their significantly better 5-year survival rates (Blows *et al.* 2010), long-term outcome of ER+ BC patients is not better because of late relapse. As these tumors represent over 70% of all BCs, most BC-related deaths are attributable to ER+ disease.

Therefore, *in vivo* models to study the biology of ER+ BC to elucidate the molecular mechanisms underlying endocrine resistance and to identify patients at risk of recurrence are urgently needed. Unfortunately, progress has been hampered by the lack of adequate *in vivo* models. Despite the similarities in the overall hormonal regulation of mammary gland development between mice and humans, the majority of the mammary carcinomas occurring in genetically engineered mouse models (GEMMs) are ER negative (ER–), and the reasons for this remain elusive. In this review, we discuss the challenges in generating ER+ BC mouse models, the lessons learned from previous efforts and the promise and challenges of using intraductal implantation of ER+ tumor cells to model the disease.

Chemically induced rodent models and genetically engineered mouse models (GEMMs)

Chemically induced rodent mammary carcinoma models developed in the 1980s have been widely used to

investigate hormone-dependent BC. Tumors are induced in rats by a single dose of oral 7,12-dimethylbenz(a)anthracene (DMBA) or intravenous or subcutaneous N-methylnitrosourea (NMU); they form with a latency between 8 and 12 weeks and a nearly 100% incidence (Russo & Russo 1996). Both NMU and DMBA-induced tumors express ER and PR (Alvarado *et al.* 2017). Administration of medroxyprogesterone acetate (MPA) decreases latency and increases the incidence of DMBA-induced tumors (Benakanakere *et al.* 2006). Importantly, these tumors faithfully recapitulate various aspects of the human disease. Their growth is hormone-dependent and pregnancy before carcinogen exposure reduces tumor incidence (Russo & Russo 1996). However, these models were not readily amenable to mechanistic studies because genetic engineering of rat models has long proven challenging. Only recently with the advent of TALEN (transcription activator-like effector nuclease) and CRISPR (clustered regularly interspersed short palindromic repeats), technology pace has picked up (Huang *et al.* 2011, Ponce de Leon *et al.* 2014). To date, most research efforts have focused on mouse models instead, which can be readily genetically manipulated. GEMMs represent an elegant tool to recapitulate *in vivo* carcinogenesis. They offer the advantage that tumors develop in the tissue of origin, in the presence of an intact immune system and organ microenvironment with stromal remodeling, inflammation and angiogenesis.

Many different GEMMs have been reported. Most of the mammary carcinomas they develop are ER negative. The histopathologies offer a variegated picture and efforts have been made to compare them to the human counterparts (Cardiff & Wellings 1999) with, in general, more squamous and mesenchymal features in the rodent models. Similarly, comparison by global gene expression profiling resulted in clusters distinct from the human subgroups (Desai *et al.* 2002, Hollern & Andrechek 2014). Altogether this begs the question of why tumorigenesis is so different between species. It may be due to inherent differences in mammary gland composition between mice and humans, and/or differences in the endocrine milieu or relate to inbreeding and/or different genetic backgrounds. Another challenge for the development of ER+ GEMMs is the lack of driver gene mutations shared by the majority of ER+ tumors. It is known that ER+ and ER– BC display distinct somatic mutation profiles. In ER+ tumors, mutations and copy number variations of multiple genes including *Pik3ca*, *PTEN*, *AKT1*, *CDH1* and *TP53* are found at varying frequencies, highlighting the intertumoral heterogeneity of BC and ER+ tumors

in particular (Nik-Zainal *et al.* 2016, Pereira *et al.* 2016). The exploitation of these driver genes for the generation of mammary epithelial-specific transgenic mice is not straightforward. As shown in mouse models expressing mutant *Pik3ca* in different mammary epithelial cell types, the same mutation can induce plasticity not seen in normal cells and result in different tumor phenotypes depending on the cell of origin (Koren *et al.* 2015, Van Keymeulen *et al.* 2015). Also, the specific developmental stages at which genetic alterations are induced might influence whether mammary tumors are ER positive. For example, Lin and colleagues reported that deletion of *P53* in prepubertal/pubertal mice, but not in adult mice, leads to the development of ER+ tumors (Lin *et al.* 2004). However, it has to be noted that in this study two different promoters were used to drive Cre recombinase-mediated deletion of *P53*, which could also affect the phenotype of the resulting tumors. Finally, the failure to reproduce the right sequence of genetic events may have a role.

Several GEMMs show some expression of ER (Dabydeen & Furth 2014) but usually lose it as tumors progress. Moreover, in order to determine whether these tumors are truly estrogen dependent like the human disease, ovariectomy or inhibition of ER signaling with the clinically applied selective ER modulator tamoxifen or selective ER degrader fulvestrant need to become a standard for the establishment of GEMMs of ER+ BC. Only few groups have performed ovariectomy or tamoxifen treatment (Frech *et al.* 2005, Zhang *et al.* 2005, Kumar *et al.* 2007, Chan *et al.* 2012, Miermont *et al.* 2012) and even fewer have found decreased tumor growth upon estrogen withdrawal (Frech *et al.* 2005, Kumar *et al.* 2007, Chan *et al.* 2012). Specific strategies were devised to generate ER+ tumors, varying from direct overexpression of ER α in mammary epithelial cells (Tilli *et al.* 2003, Miermont *et al.* 2012) to genetic alterations of molecules affecting estrogen signaling (Wang *et al.* 1994, Medina *et al.* 2002), pharmacological agents together with genetic alterations impacting estrogen signaling (Nakles *et al.* 2013), exposure to the chemical carcinogen DMBA in combination with genetic alterations (Blanco-Aparicio *et al.* 2007) and sibling matings of nude mice (Kumar *et al.* 2007).

Interestingly, encouraging results came from an unexpected model. The *Stat1*^{-/-} mice develop mammary tumors, which faithfully mimic human cancer through its progression from precursor mammary intraepithelial neoplasia to invasive ER+ cancer, show strong ER positivity and are sensitive to ovariectomy (Chan *et al.* 2012). However, because of severe concomitant effects on the immune system and mammary tumor latency of

6 months, it is not easy to use. The systemic problems can be circumvented by grafting mammary epithelium or mammary tumors to syngeneic recipients. Recently, conditional expression of *KRASG12V* in the mammary epithelium was shown to result in luminal A-like tumors that respond to anti-estrogen treatment (Ando *et al.* 2017). Cre is driven by the ovine beta lactoglobulin promoter (BLG-Cre) and the oncogenic mutation in the MMTV-driven *RAS* transgene is induced with pregnancy; consequently, tumors present within 3–9 months after lactation; it is tempting to speculate that pregnancy and lactation might be critical for generating ER+ tumors by inducing a particular differentiated cell phenotype, and this approach may be successfully exploited with other oncogenes (Ando *et al.* 2017).

Cell line xenograft models

Xenografts of human BC cell lines subcutaneously or to the mammary fat of immunocompromised mice are frequently used in preclinical drug testing. A collection of about 90 human BC cell lines, derived in some cases from primary tumors, mostly from pleural effusions, i.e. late metastatic disease, from mostly Caucasian females are currently available (ATCC 2013; Dai *et al.* 2017). Although 70% of BC are ER+ only about 30%, of the cell lines including MCF-7, T-47D, ZR-75-1, BT-474, BT-483 and MDA-MB-361 express ER (Lacroix & Leclercq 2004, Dai *et al.* 2017). In general, ER- cancer lines appear to be easier to establish *in vitro* compared to ER+ ones. It has been suggested that the secretion of extracellular matrix (ECM) proteins as well as growth factors such as EGF and TGF α and their receptors could facilitate the adherence of ER- cells to plastic and provide an autocrine loop to sustain their growth independently of exogenous growth factor supply (Ethier 1995).

Only few of the ER+ cell lines, such as MCF-7, T-47D and ZR-75-1, can be established *in vivo*. The recipient mice require supplementation with exogenous estrogen (E2) for successful growth of these xenografts (Osborne *et al.* 1984) provided through daily injection, implantation of subcutaneous pellets or supplementation of drinking water (Levin-Allerhand *et al.* 2003, Gerard *et al.* 2017). As a result estradiol levels in mice correspond to those found in premenopausal women. This is in discrepancy to the human disease, which occurs predominantly in postmenopausal women (Anderson *et al.* 2002). It has to be noted that postmenopausal women have up to four times lower estradiol levels than adult men (Greenblatt *et al.* 1976, Ismail & Barth 2001). It is important to approach

physiological estrogen levels as closely as possible because ER signaling is extremely dose dependent and high estrogen concentrations can induce differences in cancer cell behavior (Chatzistamou & Kiaris 2016). Besides the classical mechanism of action involving estrogen binding to intracellular receptors leading to their dimerization and binding to estrogen response elements (EREs) in the promoter region of target genes, estrogen also displays rapid, nongenomic actions, mediated through membrane-associated G-protein coupled receptors resulting in generation of Ca²⁺ and nitric oxide and activation of various receptor tyrosine kinase pathways (Arnal *et al.* 2017).

Different cellular responses were observed under high- or low-dose estrogens (Bolego *et al.* 1997) with low doses of 17-estradiol activating the nonclassical membrane-associated signaling (Quesada *et al.* 2002). Beneficial effects of 17-estradiol on tumor engraftment can also be attributed to their action on ER+ stromal cells leading to an improved vascularization of tumors (Pequeux *et al.* 2012).

The supraphysiological E2 levels are associated with serious adverse effects such as uterine hyperplasia and swelling of the lower reproductive tract leading to urinary retention, hydronephrosis and kidney failure (Gakhar *et al.* 2009). These factors confound the interpretation of experiments and reduce their clinical relevance.

Attempts have been made to model tumor progression starting from normal human breast epithelial cells, which were either spontaneously immortalized (Garbe *et al.* 2014), chemically transformed (Stampfer & Bartley 1985) or by lentiviral transduction with different oncogenes and the ER (Duss *et al.* 2007). A common problem is that cells derived from the normal breast epithelium lose hormone receptor expression *in vitro* and develop a basal phenotype. Concordantly, when xenografted, they show squamous differentiation and keratinization (Duss *et al.* 2007). The same group showed subsequently that injections of preneoplastic human breast epithelial cells into the milk ducts of immunodeficient mice create ER+ models that resemble luminal B adenocarcinomas resistant to fulvestrant (Verbeke *et al.* 2014). Interestingly, xenografts of the 184AA3 cell line, chemically transformed mammary cells derived from a reduction mastoplasmy (Stampfer & Bartley 1985) form ER+ cancers over the course of 1 year when injected together with irradiated fibroblasts and show histological and molecular features compatible with a luminal B subtype. They do not require E2 supplementation but estrogen depletion *in vitro* or *in vivo* does not inhibit their growth (Hines *et al.* 2016).

The difficulty to establish ER+ BC cell lines *in vivo* is a particular hurdle for studies of ILC. Compared to

invasive ductal carcinoma, which accounts for 80% of the BC cases, this special subtype is more often diagnosed in older patients and has a higher incidence of contralateral disease and gastrointestinal metastases, in particular to the ovaries and peritoneal cavity (Arpino *et al.* 2004). To date, few cell lines, such as MDA-MB-134VI, SUM-44 and IPH-926 have been established from ILC. The ER+ MDA-MB-134VI cell line, which has a large-scale deletion of the *CDH1* gene was initially reported to derive from ductal BC (Neve *et al.* 2006) and was reclassified by Reis-Filho and colleagues as ILC based on gene expression analysis (Reis-Filho *et al.* 2006). SUM-44 ER+ cells were obtained from a patient with ILC and possess a *CDH1* mutation (Ethier *et al.* 1993; <https://sumlineknowledgebase.com/sum-44-home/451-2/>). The IPH-926 ILC cell line expresses epithelial cell markers and harbors a homozygous *CDH1* mutation (Christgen *et al.* 2009). None of them has been reproducibly established as subcutaneous xenograft.

A more general limitation of GEMMs of ER+ cancer as well as ER+ cell lines established as xenografts, which extend to many other tumor models, is their limited metastatic capacity. Possibly due to the fast growth of the tumor cells at the primary site, most GEMMs and cell line xenografted mice need to be euthanized before metastatic lesions occur.

Patient-derived xenograft (PDX) models

PDXs are thought to better recapitulate human cancer biology than cell line xenografts given that prolonged passaging of human cancer cells *in vitro* possibly constitutes a selection pressure, which results in genetic drift giving rise to daughter clones distinct from the original tumors (Matthews & Sartorius 2017). PDX models rely mainly on implantation of 1 mm³ fragments derived from patient tumors into subcutaneous or mammary fat of immunocompromised mice. The tumors that develop can subsequently be passaged in mice and even after various passages *in vivo* the grafts remain phenotypically and genetically stable and maintain essential histological and molecular features of the original tumors including its metastatic potential (DeRose *et al.* 2011, Zhang *et al.* 2013a, Eirew *et al.* 2015). Moreover, PDXs show treatment responses that are comparable with the clinically obtained ones (Gao *et al.* 2015). Thus, they represent a valuable instrument for the study of tumor heterogeneity, metastasis and preclinical drug testing including signaling pathway activation before and after the occurrence of drug resistance. However, acquisition of copy number alterations during PDX passaging, possibly due to selection

of pre-existing minor clones, which differed from those appearing in patients during tumor evolution has been recently reported (Ben-David *et al.* 2017).

The most important challenge in developing ER+ PDXs is the substantially lower engraftment rate compared to that of TN or HER2+ tumors when xenografted either subcutaneously or into the mammary fat pad. In a large study where 314 ER+ tumors were engrafted, the take rate was 2.5%, compared to almost 25% for non-luminal tumors (Cottu *et al.* 2012). Particularly, there are very few PDXs derived from lower-grade and treatment-naïve ER+ luminal A tumors and lobular carcinomas. Engraftment rates of tumor samples correlate with shorter patient survival and metastatic ability (DeRose *et al.* 2011, Eyre *et al.* 2016). As a result the number of ER+ PDXs available is small (for a recent overview see Dobrolecki *et al.* (2016)).

Intraductal xenografts

Breast tumors are typically implanted into the mammary fat pad or other subcutaneous sites via surgery. The somewhat misleadingly called 'orthotopic implantation' into the mammary glands may be preferable because it is associated with a higher degree of vascularization compared to engrafting to the skin (Fleming *et al.* 2010). In both cases, artifacts are generated as the tumor cells in large numbers are placed directly into the stroma and a wound is created. The tumor cells, which are of epithelial origin and evolve *in vivo* largely surrounded by cells of their sort, with the same adhesion molecules on their surface, have to interact with stromal cells and adhere to ECM molecules, for which they do not necessarily express the receptors. Not surprisingly, most cells die; however, once tumors form, including ER+ ones supplemented with exogenous E2, they grow rapidly with a high Ki67 index not typical for hormone receptor-positive tumors.

Behbod and colleagues placed human DCIS cells, among them ER+ lesions, into the precise anatomic site where they arise from by injecting a few microliters of cell suspension into the milk ducts to study *in situ* disease in the presence of E2 pellets (Behbod *et al.* 2009, Valdez *et al.* 2011). As opposed to the 1 million cancer cells or more that are typically injected to establish tumors in the subcutaneous fat, using the intraductal method less than 10% of the cell number sufficed. The cells were initially injected into the primary milk duct visualized by surgery. Subsequent work demonstrated that cells or nanoparticles can similarly be introduced through the nipple avoiding tissue disruption (Brock *et al.* 2014, Russell *et al.* 2015).

Using this approach, we injected a panel of BC cell lines representative of different subtypes directly into the milk ducts of immunocompromised mice and showed that all of them grew without E2 supplementation (Sflomos *et al.* 2016). Basal-like cell lines gave rise to palpable tumors as early as 3 weeks after injection and presented as highly vascularized and invasive tumors upon macroscopic and histological analysis. The HER2-overexpressing BT474 showed characteristic comedo features while luminal cell lines went through an extended *in situ* phase and distended the milk ducts but barely affected the size of the mammary gland (Sflomos *et al.* 2016). Blood vessels were sparse around distended milk ducts and the Ki67 index came close to that of human ER+ BCs. Comparison of MCF-7 cells established in the fat pad and intraductally by global gene expression profiling showed that mammary stroma induces TGF β /SLUG signaling leading to basal differentiation, whereas the intraductal microenvironment enhances expression of ER, androgen receptor (AR) and growth hormone receptor (GR) and other receptors important in mammary physiology (Sflomos *et al.* 2016). In contrast, basal-like BC cell lines did not show any significant gene expression changes related to the two distinct anatomic sites. Thus, the intraductal microenvironment is a determinant of hormone-sensitive/luminal breast cancer cell phenotype, not only does it enable these cells to establish themselves but it allows them to behave like in their native environment. Indeed, the intraductal tumors closely resemble the original tumors regarding histological characteristics, hormone receptor expression and even the formation of microcalcifications, a clinical hallmark of human BC that is not observed when tumors are implanted in other anatomic sites. Importantly, the course of the disease is largely recapitulated; spontaneous micrometastases are found in clinically relevant organs such as brain, bones, lungs and liver (Sflomos *et al.* 2016). Furthermore, the ILC cell line MDA-MB-134VI, successfully grew when injected intraductally opening new opportunities for *in vivo* studies of this special subtype (Sflomos *et al.* 2016) (Fig. 1).

ER+ tumor cells isolated from fresh surgical samples engrafted readily by the intraductal approach with rates between 30 and 100%, indicating the number of successfully engrafted mammary glands per tumor. Take rates were independent of tumor grade and tumor subtypes and included lobular carcinomas (Sflomos *et al.* 2016). Similarly, molecular apocrine, AR-positive breast tumors grew with a similarly high success rate when tumors engrafted intraductally (Richard *et al.* 2016).

Figure 1

Modeling of hormone receptor-positive breast cancer in mice. Scheme showing the mammary fat pad vs intraductal xenograft approach; tumor cells isolated from surgically resected breast carcinomas are injected either into the fat pad or the lumina of the milk ducts. In the fat pad, tumor cells interact with adipocytes, fibroblasts and other stromal cells and require exogenous estrogen supplementation for growth. Under these conditions, activation of TGF β /SLUG signaling leads to basal differentiation. Cancer cells that are grafted to the milk ducts will establish themselves in the mouse mammary epithelium. In the ductal microenvironment ER+ tumor cells retain cell polarity along with high level ER expression without exogenous estradiol supplementation. Tumors form in the milk ducts that recapitulate the human disease.

Both cell line- and patient derived-xenografts responded in predicted fashion to chemotherapy and endocrine treatments. This means that *in vivo* treatments can now be applied for ER+ BC under physiological postmenopausal hormonal levels, and the studies can be extended for several months without the survival of the mice being compromised due to E2-related adverse effects (Collins *et al.* 2017).

Remaining challenges and outlook

The key limitation of intraductal xenograft models is that they are established in severely immunodeficient mice lacking T and B lymphocytes and a fully functional innate

immune system (Valdez *et al.* 2011). To what extent the lack of a normal immune response affects ER+ breast carcinogenesis at different stages is not understood. The introduction of immune checkpoint inhibitors targeting CTLA4 or the PD1/PD-L1 axis has been a game changer in the treatment of various metastatic cancers and provides for the first time durable therapy responses in about 15–20% of the patients (Sharma & Allison 2015). To overcome this substantial hurdle in PDX research and to allow researchers to assess critical interactions of therapies with the immune system, a lot of effort is placed in the generation of humanized mouse models for cancer, which promises to improve the utility of the models.

Three main strategies are employed to establish a human immune system in immunodeficient IL2Rgamma-null mice (Walsh *et al.* 2017). The first model, HU-PBL-SCID (human peripheral blood leucocytes-SCID) mouse is based on the injection of human peripheral blood mononuclear cells (PBMC) resulting in the engraftment of human CD3+ T cells after 1 week. It was originally developed for the study of graft vs host disease (GvHD), which appears within 1–2 months, providing only a short experimental window (King *et al.* 2009). The second model, Hu-SRC (human-SCID repopulating) mouse, comprises the transplantation of CD34+ human hematopoietic stem cells (HSCs) obtained either from the bone marrow or mobilized from the peripheral blood using granulocyte colony-stimulating factor (G-CSF), leading to engraftment of a complete human immune system. The drawbacks include that the human T cells are educated in the mouse thymus and are thus H2 and not HLA-restricted, limiting the recognition of human cancer cells (Watanabe *et al.* 2009). However, human BC xenografts presenting an immune cell infiltrate using HU-SRC-SCID mice have been described (Wege *et al.* 2011). The third model, the bone marrow/liver/thymus (BLT) mouse, is a modification of the HU-SRC model and represents the most robust human immune system engraftment model available. It is generated through transplantation of human fetal liver and thymus under the kidney capsule and the intravenous injection of human fetal liver HSC, giving rise to all lineages of human immune cells (Lan *et al.* 2006, Melkus *et al.* 2006). It has the advantage that the human T cells are educated in the human thymus and therefore HLA restricted, and it develops a functional mucosal immunity. Again this model develops a severe GvHD-like syndrome 5–6 months after reconstitution and is susceptible to thymic lymphoma (Greenblatt *et al.* 2012).

Generation of ER+ PDX and xenografts in humanized mice, which contain a functional human immune system could substantially improve our understanding of the crosstalk between the host immune system and ER+ cancers, allow preclinical researchers to test immunotherapies and evaluate new therapies in the context of an immune response. However, we need to keep in mind that humanized mice are currently not fully capable of reflecting human immune responses, due to differences in the relative frequency of the reconstituted immune cell types (e.g. granulocytes account for 65–75% of the leukocytes in human blood but for 2–3% in humanized mice) and the insufficient differentiation and maturation of the human cells in the presence of mouse

cytokines (Ito *et al.* 2012). Plasmid-mediated expression or knockin of human genes encoding cytokines improves the differentiation and function of innate immune cells from human fetal liver or adult stem cells grafted into mice (Chen *et al.* 2009, Rongvaux *et al.* 2014).

Although the intraductal model overcomes the requirement for exogenous E2 substitution for the establishment of ER+ xenografts/PDX further adjustments of the mouse endogenous hormone levels will be required to study the behavior of cancer cells under physiological hormone concentrations. The hormonal milieu of a mouse with recurrent estrous cycles is not identical to that of the postmenopausal patient. Furthermore, an important part of BCs and their pathogenesis occurs in premenopausal women, mimicking the premenopausal endocrine milieu in mice with its cyclic changes is yet another important challenge to address.

Other limitations of the model relate to specificities of the murine vs human species. A critical one concerns aromatase, the enzyme that catalyzes the conversion of androgens into estrogens. Currently, treatment for at least 5 years with aromatase inhibitors represents the cornerstone of ER+ BC therapy in postmenopausal women and significantly reduces risk of recurrence (Early Breast Cancer Trialists' Collaborative Group 2015, Goss *et al.* 2016). Mouse models overexpressing aromatase driven by the MMTV promoter (int-5/aromatase) (Tekmal *et al.* 1996) and xenografts using aromatase-overexpressing MFC-7 cells confirmed the essential role of locally produced estrogens in fueling the growth of ER+ mammary cancers and lead to the development of aromatase inhibitors in the first place (Brodie *et al.* 1999, Brodie *et al.* 2003). However, the human gene has at least 10 different tissue-specific first exons that account for aromatase expression in many peripheral organs (Harada *et al.* 1993) while mice express aromatase only in gonads and brain (Golovine *et al.* 2003). Mice with broader, more human-like aromatase expression in different tissues were developed (Zhao *et al.* 2012) but do not reproduce the complexities of the expression in human tissues. Hence, the use of mice as a preclinical model to study aromatase inhibition is somewhat limited.

Notwithstanding these weaknesses, the intraductal ER+ BC xenograft models offer new opportunities for preclinical studies. Targeting of alternative signaling pathways such as MAPK, EGFR and PI3K pathways contributing to endocrine escape needs to be performed in presence of estrogen levels, which reflect the levels found in ER+ BC patients. Furthermore, understanding the interplay between growth factors and estrogen signaling

has important clinical consequences as illustrated by the development of cyclin-dependent kinase (CDK) inhibitors (Finn *et al.* 2016). The CDK4/6 inhibitor palbociclib in combination with antiestrogens improves significantly the progression-free survival of women with metastatic ER+ BC irrespective of the degree of endocrine resistance and expression levels of ER or PR and represents the new standard first- and second-line therapy for these patients (Cristofanilli *et al.* 2016). However, resistance to palbociclib as a result of cyclin E (*CCNE1*) overexpression and loss of *RB1* has been reported and the evaluation of novel combination therapies and exploration of biomarkers predicting sensitivity or resistance to CDK4/6-inhibition requires *in vivo* models that closely recapitulate human ER+ cancers (Herrera-Abreu *et al.* 2016).

Advances can also be expected for our understanding of late disease relapse with predominant bone metastasis, which accounts for the majority of cancer-related deaths. MCF-7 grafted intraductally recapitulate the metastatic pattern of a typical ER+ NST and may turn out useful to study the cellular and molecular mechanisms underlying dormancy. Again with currently the caveat that the use of severely immunocompromised mice hampers the study of the role of the immune system in cancer cell dormancy.

Male BC is a rare disease comprising 1% of all BC cases. ER+ ductal carcinoma is by far the commonest subtype (>90%). Risk factors include hyperestrogenization due to Klinefelter syndrome, gonadal dysfunction and obesity, although most men with BC present none of these risk factors. Due to the rarity of male BC, the current understanding of the pathogenesis and its treatment are largely based on extrapolation of data from female BC patients (Fentiman *et al.* 2006). However, emerging evidence suggests significant sex differences in the regulation of major signaling pathways, in tissue functions such as immune cell recruitment and ECM remodeling, and in response to endocrine therapies (Fentiman 2016). Mouse models of male BC are therefore required to study this particular disease in its tissue of origin and appropriate hormonal milieu and develop tailored therapies taking into account differences rather than similarities with female BC.

A final challenge for GEMMs and xenograft models is the development of liquid biopsies and biomarkers for ER+ BC. CA 15-3 is the most widely used biomarker with prognostic significance (Shao *et al.* 2015). During the last years, liquid biopsies referring to the analysis of circulating tumor cells (CTCs) and cell-free circulating tumor DNA (ctDNA) have been developed as surrogate markers to inform on tumor heterogeneity, depict real-time tumor

genomic evolution, identify developing resistance mechanisms and measure disease burden (Cristofanilli *et al.* 2005b, Dawson *et al.* 2013, Aceto *et al.* 2014). Therefore, the miniaturization of diagnostic procedures are required to handle the minute blood samples obtained from mice and perform assays for the detection of CTCs and ctDNA in mouse models.

For individuals interested in using the intraductal approach a course is offered, information can be found at <https://briskens-lab.epfl.ch/PreclinicalModelCourse>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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References

- Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Wittner BS, Spencer JA, Yu M, Pely A, Engstrom A, Zhu H, *et al.* 2014 Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. *Cell* **158** 1110–1122. (<https://doi.org/10.1016/j.cell.2014.07.013>)
- Alvarado A, Lopes AC, Faustino-Rocha AI, Cabrita AMS, Ferreira R, Oliveira PA & Colaco B 2017 Prognostic factors in MNU and DMBA-induced mammary tumors in female rats. *Pathology, Research and Practice* **213** 441–446. (<https://doi.org/10.1016/j.prp.2017.02.014>)
- Anderson WF, Chatterjee N, Ershler WB & Brawley OW 2002 Estrogen receptor breast cancer phenotypes in the surveillance, epidemiology, and end results database. *Breast Cancer Research and Treatment* **76** 27–36. (<https://doi.org/10.1023/A:1020299707510>)
- Ando S, Malivindi R, Catalano S, Rizza P, Barone I, Panza S, Rovito D, Emprou C, Bornert JM, Laverny G, *et al.* 2017 Conditional expression of Ki-Ras(G12V) in the mammary epithelium of transgenic mice induces estrogen receptor alpha (ERalpha)-positive adenocarcinoma. *Oncogene* **36** 6420–6431. (<https://doi.org/10.1038/onc.2017.252>)
- Arnal JF, Lenfant F, Metivier R, Flouriot G, Henrion D, Adlanmerini M, Fontaine C, Gourdy P, Chambon P, Katzenellenbogen B, *et al.* 2017 Membrane and nuclear estrogen receptor alpha actions: from tissue specificity to medical implications. *Physiological Reviews* **97** 1045–1087. (<https://doi.org/10.1152/physrev.00024.2016>)
- Arpino G, Bardou VJ, Clark GM & Elledge RM 2004 Infiltrating lobular carcinoma of the breast: tumor characteristics and clinical outcome. *Breast Cancer Research* **6** R149–R156. (<https://doi.org/10.1186/bcr767>)
- ATCC 2013 Breast cancer and normal cell lines. Manassas, VA, USA: American Type Culture Collection. (available at: <https://www.atcc.org/~media/PDFs/Cancer%20and%20Normal%20cell%20lines%20tables/Breast%20cancer%20and%20normal%20cell%20lines.ashx>)
- Behbod F, Kittrell FS, LaMarca H, Edwards D, Kerbawy S, Heestand JC, Young E, Mukhopadhyay P, Yeh HW, Allred DC, *et al.* 2009 An

- intraductal human-in-mouse transplantation model mimics the subtypes of ductal carcinoma in situ. *Breast Cancer Research* **11** R66. (<https://doi.org/10.1186/bcr2358>)
- Ben-David U, Ha G, Tseng YY, Greenwald NF, Oh C, Shih J, McFarland JM, Wong B, Boehm JS, Beroukhim R, *et al.* 2017 Patient-derived xenografts undergo mouse-specific tumor evolution. *Nature Genetics* **49** 1567–1575. (<https://doi.org/10.1038/ng.3967>)
- Benakanakere I, Besch-Williford C, Schnell J, Brandt S, Ellersieck MR, Molinolo A & Hyder SM 2006 Natural and synthetic progestins accelerate 7,12-dimethylbenz[a]anthracene-initiated mammary tumors and increase angiogenesis in Sprague-Dawley rats. *Clinical Cancer Research* **12** 4062–4071. (<https://doi.org/10.1158/1078-0432.CCR-06-0427>)
- Bentzon N, During M, Rasmussen BB, Mouridsen H & Kroman N 2008 Prognostic effect of estrogen receptor status across age in primary breast cancer. *International Journal of Cancer* **122** 1089–1094. (<https://doi.org/10.1002/ijc.22892>)
- Blanco-Aparicio C, Perez-Gallego L, Pequeno B, Leal JF, Renner O & Carnero A 2007 Mice expressing myrAKT1 in the mammary gland develop carcinogen-induced ER-positive mammary tumors that mimic human breast cancer. *Carcinogenesis* **28** 584–594. (<https://doi.org/10.1093/carcin/bgl190>)
- Blows FM, Driver KE, Schmidt MK, Broeks A, van Leeuwen FE, Wesseling J, Cheang MC, Gelmon K, Nielsen TO, Blomqvist C, *et al.* 2010 Subtyping of breast cancer by immunohistochemistry to investigate a relationship between subtype and short and long term survival: a collaborative analysis of data for 10,159 cases from 12 studies. *PLoS Medicine* **7** e1000279. (<https://doi.org/10.1371/journal.pmed.1000279>)
- Bolego C, Cignarella A, Ruzza R, Zaarour C, Messi E, Zanisi M & Puglisi L 1997 Differential effects of low- and high-dose estrogen treatments on vascular responses in female rats. *Life Sciences* **60** 2291–2302. ([https://doi.org/10.1016/S0024-3205\(97\)00284-1](https://doi.org/10.1016/S0024-3205(97)00284-1))
- Brock A, Krause S, Li H, Kowalski M, Goldberg MS, Collins JJ & Ingber DE 2014 Silencing HoxA1 by intraductal injection of siRNA lipidoid nanoparticles prevents mammary tumor progression in mice. *Science Translational Medicine* **6** 217–212. (<https://doi.org/10.1126/scitranslmed.3007048>)
- Brodie A, Lu Q, Liu Y & Long B 1999 Aromatase inhibitors and their antitumor effects in model systems. *Endocrine-Related Cancer* **6** 205–210. (<https://doi.org/10.1677/erc.0.0060205>)
- Brodie A, Jelovac D & Long BJ 2003 Predictions from a preclinical model: studies of aromatase inhibitors and antiestrogens. *Clinical Cancer Research* **9** 455S–459S.
- Cardiff RD & Wellings SR 1999 The comparative pathology of human and mouse mammary glands. *Journal of Mammary Gland Biology and Neoplasia* **4** 105–122. (<https://doi.org/10.1023/A:1018712905244>)
- Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, Karaca G, Troester MA, Tse CK, Edmiston S, *et al.* 2006 Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *JAMA* **295** 2492–2502. (<https://doi.org/10.1001/jama.295.21.2492>)
- Chan SR, Vermi W, Luo J, Lucini L, Rickert C, Fowler AM, Lonardi S, Arthur C, Young LJ, Levy DE, *et al.* 2012 STAT1-deficient mice spontaneously develop estrogen receptor alpha-positive luminal mammary carcinomas. *Breast Cancer Research* **14** R16. (<https://doi.org/10.1186/bcr3100>)
- Chatzistamou I & Kiaris H 2016 Modeling estrogen receptor-positive breast cancers in mice: is it the best we can do? *Endocrine-Related Cancer* **23** C9–C12. (<https://doi.org/10.1530/ERC-16-0397>)
- Chen Q, Khoury M & Chen J 2009 Expression of human cytokines dramatically improves reconstitution of specific human-blood lineage cells in humanized mice. *PNAS* **106** 21783–21788. (<https://doi.org/10.1073/pnas.0912274106>)
- Christgen M, Bruchhardt H, Hadamitzky C, Rudolph C, Steinemann D, Gadzicki D, Hasemeier B, Romermann D, Focken T, Krech T, *et al.* 2009 Comprehensive genetic and functional characterization of IPH-926: a novel CDH1-null tumour cell line from human lobular breast cancer. *Journal of Pathology* **217** 620–632. (<https://doi.org/10.1002/path.2495>)
- Collins DE, Mulka KR, Hoenerhoff MJ, Taichman RS & Villano JS 2017 Clinical assessment of urinary tract damage during sustained-release estrogen supplementation in mice. *Comparative Medicine* **67** 11–21.
- Cottu P, Marangoni E, Assayag F, de Cremoux P, Vincent-Salomon A, Guyader C, de Plater L, Elbaz C, Karboul N, Fontaine JJ, *et al.* 2012 Modeling of response to endocrine therapy in a panel of human luminal breast cancer xenografts. *Breast Cancer Research and Treatment* **133** 595–606. (<https://doi.org/10.1007/s10549-011-1815-5>)
- Cristofanilli M, Gonzalez-Angulo A, Sneige N, Kau SW, Broglio K, Theriault RL, Valero V, Buzdar AU, Kuerer H, Buchholz TA, *et al.* 2005a Invasive lobular carcinoma classic type: response to primary chemotherapy and survival outcomes. *Journal of Clinical Oncology* **23** 41–48. (<https://doi.org/10.1200/JCO.2005.03.111>)
- Cristofanilli M, Hayes DF, Budd GT, Ellis MJ, Stopeck A, Reuben JM, Doyle GV, Matera J, Allard WJ, Miller MC, *et al.* 2005b Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer. *Journal of Clinical Oncology* **23** 1420–1430. (<https://doi.org/10.1200/JCO.2005.08.140>)
- Cristofanilli M, Turner NC, Bondarenko I, Ro J, Im SA, Masuda N, Colleoni M, DeMichele A, Loi S, Verma S, *et al.* 2016 Fulvestrant plus palbociclib versus fulvestrant plus placebo for treatment of hormone-receptor-positive, HER2-negative metastatic breast cancer that progressed on previous endocrine therapy (PALOMA-3): final analysis of the multicentre, double-blind, phase 3 randomised controlled trial. *Lancet Oncology* **17** 425–439. ([https://doi.org/10.1016/S1470-2045\(15\)00613-0](https://doi.org/10.1016/S1470-2045(15)00613-0))
- Dabydeen SA & Furth PA 2014 Genetically engineered ERalpha-positive breast cancer mouse models. *Endocrine-Related Cancer* **21** R195–R208. (<https://doi.org/10.1530/ERC-13-0512>)
- Dai X, Cheng H, Bai Z & Li J 2017 Breast cancer cell line classification and its relevance with breast tumor subtyping. *Journal of Cancer* **8** 3131–3141. (<https://doi.org/10.7150/jca.18457>)
- Dawson SJ, Tsui DW, Murtaza M, Biggs H, Rueda OM, Chin SF, Dunning MJ, Gale D, Forshew T, Mahler-Araujo B, *et al.* 2013 Analysis of circulating tumor DNA to monitor metastatic breast cancer. *New England Journal of Medicine* **368** 1199–1209. (<https://doi.org/10.1056/NEJMoa1213261>)
- DeRose YS, Wang G, Lin YC, Bernard PS, Buys SS, Ebbert MT, Factor R, Matsen C, Milash BA, Nelson E, *et al.* 2011 Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. *Nature Medicine* **17** 1514–1520. (<https://doi.org/10.1038/nm.2454>)
- Desai KV, Xiao N, Wang W, Gangi L, Greene J, Powell JJ, Dickson R, Furth P, Hunter K, Kucherlapati R, *et al.* 2002 Initiating oncogenic event determines gene-expression patterns of human breast cancer models. *PNAS* **99** 6967–6972. (<https://doi.org/10.1073/pnas.102172399>)
- Dobrolecki LE, Airhart SD, Alferez DG, Aparicio S, Behbod F, Bentes-Alj M, Briskin C, Bult CJ, Cai S, Clarke RB, *et al.* 2016 Patient-derived xenograft (PDX) models in basic and translational breast cancer research. *Cancer and Metastasis Reviews* **35** 547–573. (<https://doi.org/10.1007/s10555-016-9653-x>)
- Duss S, Andre S, Nicoulaz AL, Fiche M, Bonnefoi H, Briskin C & Iggo RD 2007 An oestrogen-dependent model of breast cancer created by transformation of normal human mammary epithelial cells. *Breast Cancer Research* **9** R38. (<https://doi.org/10.1186/bcr1734>)
- Early Breast Cancer Trialists' Collaborative Group 2015 Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. *Lancet* **386** 1341–1352. ([https://doi.org/10.1016/S0140-6736\(15\)61074-1](https://doi.org/10.1016/S0140-6736(15)61074-1))
- Eirew P, Steif A, Khattri J, Ha G, Yap D, Farahani H, Gelmon K, Chia S, Mar C, Wan A, *et al.* 2015 Dynamics of genomic clones in breast

- cancer patient xenografts at single-cell resolution. *Nature* **518** 422–426. (<https://doi.org/10.1038/nature13952>)
- Ethier SP 1995 Growth factor synthesis and human breast cancer progression. *Journal of the National Cancer Institute* **87** 964–973. (<https://doi.org/10.1093/jnci/87.13.964>)
- Ethier SP, Mahacek ML, Gullick WJ, Frank TS & Weber BL 1993 Differential isolation of normal luminal mammary epithelial cells and breast cancer cells from primary and metastatic sites using selective media. *Cancer Research* **53** 627–635.
- Eyre R, Alferez DG, Spence K, Kamal M, Shaw FL, Simoes BM, Santiago-Gomez A, Sarmiento-Castro A, Bramley M, Absar M, *et al.* 2016 Patient-derived mammosphere and xenograft tumour initiation correlates with progression to metastasis. *Journal of Mammary Gland Biology and Neoplasia* **21** 99–109. (<https://doi.org/10.1007/s10911-016-9361-8>)
- Fentiman IS 2016 Male breast cancer is not congruent with the female disease. *Critical Reviews in Oncology/Hematology* **101** 119–124. (<https://doi.org/10.1016/j.critrevonc.2016.02.017>)
- Fentiman IS, Fourquet A & Hortobagyi GN 2006 Male breast cancer. *Lancet* **367** 595–604. ([https://doi.org/10.1016/S0140-6736\(06\)68226-3](https://doi.org/10.1016/S0140-6736(06)68226-3))
- Finn RS, Aleshin A & Slamon DJ 2016 Targeting the cyclin-dependent kinases (CDK) 4/6 in estrogen receptor-positive breast cancers. *Breast Cancer Research* **18** 17. (<https://doi.org/10.1186/s13058-015-0661-5>)
- Fleming JM, Miller TC, Meyer MJ, Ginsburg E & Vonderhaar BK 2010 Local regulation of human breast xenograft models. *Journal of Cellular Physiology* **224** 795–806. (<https://doi.org/10.1002/jcp.22190>)
- Frech MS, Halama ED, Tilli MT, Singh B, Gunther EJ, Chodosh LA, Flaws JA & Furth PA 2005 Dereglated estrogen receptor alpha expression in mammary epithelial cells of transgenic mice results in the development of ductal carcinoma in situ. *Cancer Research* **65** 681–685.
- Gakhar G, Wight-Carter M, Andrews G, Olson S, Nguyen TA 2009 Hydronephrosis and urine retention in estrogen-implanted athymic nude mice. *Veterinary Pathology* **46** 505–508. (<https://doi.org/10.1354/vp.08-VP-0180-N-BC>)
- Gao H, Korn JM, Ferretti S, Monahan JE, Wang Y, Singh M, Zhang C, Schnell C, Yang G, Zhang Y, *et al.* 2015 High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. *Nature Medicine* **21** 1318–1325. (<https://doi.org/10.1038/nm.3954>)
- Garbe JC, Vrba L, Sputova K, Fuchs L, Novak P, Brothman AR, Jackson M, Chin K, LaBarge MA, Watts G, *et al.* 2014 Immortalization of normal human mammary epithelial cells in two steps by direct targeting of senescence barriers does not require gross genomic alterations. *Cell Cycle* **13** 3423–3435. (<https://doi.org/10.4161/15384101.2014.954456>)
- Gerard C, Gallez A, Dubois C, Drion P, Delahaut P, Quertemont E, Noel A & Pequeux C 2017 Accurate control of 17beta-estradiol long-term release increases reliability and reproducibility of preclinical animal studies. *Journal of Mammary Gland Biology and Neoplasia* **22** 1–11. (<https://doi.org/10.1007/s10911-016-9368-1>)
- Golovine K, Schwerin M & Vanselow J 2003 Three different promoters control expression of the aromatase cytochrome p450 gene (cyp19) in mouse gonads and brain. *Biology of Reproduction* **68** 978–984. (<https://doi.org/10.1095/biolreprod.102.008037>)
- Goss PE, Ingle JN, Pritchard KI, Robert NJ, Muss H, Gralow J, Gelmon K, Whelan T, Strasser-Weippl K, Rubin S, *et al.* 2016 Extending aromatase-inhibitor adjuvant therapy to 10 years. *New England Journal of Medicine* **375** 209–219. (<https://doi.org/10.1056/NEJMoa1604700>)
- Greenblatt RB, Oettinger M & Bohler CS 1976 Estrogen-androgen levels in aging men and women: therapeutic considerations. *Journal of the American Geriatrics Society* **24** 173–178. (<https://doi.org/10.1111/j.1532-5415.1976.tb04294.x>)
- Greenblatt MB, Vrbancac V, Tivey T, Tsang K, Tager AM & Aliprantis AO 2012 Graft versus host disease in the bone marrow, liver and thymus humanized mouse model. *PLoS ONE* **7** e44664. (<https://doi.org/10.1371/journal.pone.0044664>)
- Harada N, Utsumi T & Takagi Y 1993 Tissue-specific expression of the human aromatase cytochrome P-450 gene by alternative use of multiple exons 1 and promoters, and switching of tissue-specific exons 1 in carcinogenesis. *PNAS* **90** 11312–11316. (<https://doi.org/10.1073/pnas.90.23.11312>)
- Herrera-Abreu MT, Palafox M, Asghar U, Rivas MA, Cutts RJ, Garcia-Murillas I, Pearson A, Guzman M, Rodriguez O, Grueso J, *et al.* 2016 Early adaptation and acquired resistance to CDK4/6 inhibition in estrogen receptor-positive breast cancer. *Cancer Research* **76** 2301–2313. (<https://doi.org/10.1158/0008-5472.CAN-15-0728>)
- Hines WC, Kuhn I, Thi K, Chu B, Stanford-Moore G, Sampayo R, Garbe JC, Stampfer M, Borowsky AD & Bissell MJ 2016 184AA3: a xenograft model of ER+ breast adenocarcinoma. *Breast Cancer Research and Treatment* **155** 37–52. (<https://doi.org/10.1007/s10549-015-3649-z>)
- Hollern DP & Andrechek ER 2014 A genomic analysis of mouse models of breast cancer reveals molecular features of mouse models and relationships to human breast cancer. *Breast Cancer Research* **16** R59. (<https://doi.org/10.1186/bcr3672>)
- Huang G, Ashton C, Kumbhani DS & Ying QL 2011 Genetic manipulations in the rat: progress and prospects. *Current Opinion in Nephrology and Hypertension* **20** 391–399. (<https://doi.org/10.1097/MNH.0b013e328347768a>)
- Ismail AA & Barth JH 2001 Endocrinology of gynaecomastia. *Annals of Clinical Biochemistry* **38** 596–607. (<https://doi.org/10.1258/0004563011900993>)
- Ito R, Takahashi T, Katano I & Ito M 2012 Current advances in humanized mouse models. *Cellular and Molecular Immunology* **9** 208–214. (<https://doi.org/10.1038/cmi.2012.2>)
- King MA, Covassin L, Brehm MA, Racki W, Pearson T Leif J, Laning J, Fodor W, Foreman O, Burzenski L, *et al.* 2009 Human peripheral blood leucocyte non-obese diabetic-severe combined immunodeficiency interleukin-2 receptor gamma chain gene mouse model of xenogeneic graft-versus-host-like disease and the role of host major histocompatibility complex. *Clinical and Experimental Immunology* **157** 104–118. (<https://doi.org/10.1111/j.1365-2249.2009.03933.x>)
- Koren S, Reavie L, Couto JP, De Silva D, Stadler MB, Roloff T, Britschgi A, Eichlisberger T, Kohler H, Aina O, *et al.* 2015 PIK3CA(H1047R) induces multipotency and multi-lineage mammary tumours. *Nature* **525** 114–118. (<https://doi.org/10.1038/nature14669>)
- Kumar MJ, Ponvijay KS, Nandhini R, Nagarajan RS, Jose J, Srinivas G, Nagarajan P, Venkatesan R, Kumar K & Singh S 2007 A mouse model for luminal epithelial like ER positive subtype of human breast cancer. *BMC Cancer* **7** 180. (<https://doi.org/10.1186/1471-2407-7-180>)
- Lacroix M & Leclercq G 2004 Relevance of breast cancer cell lines as models for breast tumours: an update. *Breast Cancer Research and Treatment* **83** 249–289. (<https://doi.org/10.1023/B:BREA.0000014042.54925.cc>)
- Lan P, Tonomura N, Shimizu A, Wang S & Yang YG 2006 Reconstitution of a functional human immune system in immunodeficient mice through combined human fetal thymus/liver and CD34+ cell transplantation. *Blood* **108** 487–492. (<https://doi.org/10.1182/blood-2005-11-4388>)
- Levin-Allerhand JA, Sokol K & Smith JD 2003 Safe and effective method for chronic 17beta-estradiol administration to mice. *Contemporary Topics in Laboratory Animal Science* **42** 33–35.
- Lin SC, Lee KF, Nikitin AY, Hilsenbeck SG, Cardiff RD, Li A, Kang KW, Frank SA, Lee WH & Lee EY 2004 Somatic mutation of p53 leads to estrogen receptor alpha-positive and -negative mouse mammary tumors with high frequency of metastasis. *Cancer Research* **64** 3525–3532. (<https://doi.org/10.1158/0008-5472.CAN-03-3524>)
- Matthews SB & Sartorius CA 2017 Steroid hormone receptor positive breast cancer patient-derived xenografts. *Hormones and Cancer* **8** 4–15. (<https://doi.org/10.1007/s12672-016-0275-0>)

- Medina D, Kittrell FS, Shepard A, Stephens LC, Jiang C, Lu J, Allred DC, McCarthy M & Ullrich RL 2002 Biological and genetic properties of the p53 null preneoplastic mammary epithelium. *FASEB Journal* **16** 881–883. (<https://doi.org/10.1096/fj.01-0885fje>)
- Melkus MW, Estes JD, Padgett-Thomas A, Gatlin J, Denton PW, Othieno FA, Wege AK, Haase AT & Garcia JV 2006 Humanized mice mount specific adaptive and innate immune responses to EBV and TSST-1. *Nature Medicine* **12** 1316–1322. (<https://doi.org/10.1038/nm1431>)
- Miermont AM, Cabrera MC, Frech SM, Nakles RE, Diaz-Cruz ES, Shiffert MT & Furth PA 2012 Association of over-expressed estrogen receptor alpha with development of tamoxifen resistant hyperplasia and adenocarcinomas in genetically engineered mice. *Anatomy and Physiology* (Supplement 12).
- Murphy CG & Dickler MN 2016 Endocrine resistance in hormone-responsive breast cancer: mechanisms and therapeutic strategies. *Endocrine-Related Cancer* **23** R337–R352. (<https://doi.org/10.1530/ERC-16-0121>)
- Nakles RE, Kallakury BV & Furth PA 2013 The PPARGgamma agonist efatutazone increases the spectrum of well-differentiated mammary cancer subtypes initiated by loss of full-length BRCA1 in association with TP53 haploinsufficiency. *American Journal of Pathology* **182** 1976–1985. (<https://doi.org/10.1016/j.ajpath.2013.02.006>)
- Neve RM, Chin K, Fridlyand J, Yeh J, Baehner FL, Fevr T, Clark L, Bayani N, Coppe JP, Tong F, *et al.* 2006 A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. *Cancer Cell* **10** 515–527. (<https://doi.org/10.1016/j.ccr.2006.10.008>)
- Nik-Zainal S, Davies H, Staaf J, Ramakrishna M, Glodzik D, Zou X, Martincorena I, Alexandrov LB, Martin S, Wedge DC, *et al.* 2016 Landscape of somatic mutations in 560 breast cancer whole-genome sequences. *Nature* **534** 47–54. (<https://doi.org/10.1038/nature17676>)
- Osborne CK, Boldt DH & Estrada P 1984 Human breast cancer cell cycle synchronization by estrogens and antiestrogens in culture. *Cancer Research* **44** 1433–1439.
- Pequeux C, Raymond-Letron I, Blacher S, Boudou F, Adlanmerini M, Fouque MJ, Rochaix P, Noel A, Foidart JM, Krust A, *et al.* 2012 Stromal estrogen receptor-alpha promotes tumor growth by normalizing an increased angiogenesis. *Cancer Research* **72** 3010–3019. (<https://doi.org/10.1158/0008-5472.CAN-11-3768>)
- Pereira B, Chin SE, Rueda OM, Vollan HK, Provenzano E, Bardwell HA, Pugh M, Jones L, Russell R, Sammut SJ, *et al.* 2016 The somatic mutation profiles of 2,433 breast cancers refines their genomic and transcriptomic landscapes. *Nature Communications* **7** 11479. (<https://doi.org/10.1038/ncomms11479>)
- Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, *et al.* 2000 Molecular portraits of human breast tumours. *Nature* **406** 747–752. (<https://doi.org/10.1038/35021093>)
- Ponce de Leon V, Merillat AM, Tesson L, Anegón, I & Hummler E 2014 Generation of TALEN-mediated GRdim knock-in rats by homologous recombination. *PLoS ONE* **9** e88146. (<https://doi.org/10.1371/journal.pone.0088146>)
- Quesada I, Fuentes E, Viso-Leon MC, Soria B, Ripoll C & Nadal A 2002 Low doses of the endocrine disruptor bisphenol-A and the native hormone 17beta-estradiol rapidly activate transcription factor CREB. *FASEB Journal* **16** 1671–1673. (<https://doi.org/10.1096/fj.02-0313fje>)
- Reis-Filho JS, Simpson PT, Turner NC, Lambros MB, Jones C, Mackay A, Grigoriadis A, Sarrio D, Savage K, Dexter T, *et al.* 2006 FGFR1 emerges as a potential therapeutic target for lobular breast carcinomas. *Clinical Cancer Research* **12** 6652–6662. (<https://doi.org/10.1158/1078-0432.CCR-06-1164>)
- Richard E, Grellety T, Velasco V, MacGrogan G, Bonnefoi H & Iggo R 2016 The mammary ducts create a favourable microenvironment for xenografting of luminal and molecular apocrine breast tumours. *Journal of Pathology* **240** 256–261. (<https://doi.org/10.1002/path.4772>)
- Rongvaux A, Willinger T, Martinek J, Strowig T, Gearty SV, Teichmann LL, Saito Y, Marches F, Halene S, Palucka AK, *et al.* 2014 Development and function of human innate immune cells in a humanized mouse model. *Nature Biotechnology* **32** 364–372. (<https://doi.org/10.1038/nbt.2858>)
- Russell TD, Jindal S, Agunbiade S, Gao D, Troxell M, Borges VF & Schedin P 2015 Myoepithelial cell differentiation markers in ductal carcinoma in situ progression. *American Journal of Pathology* **185** 3076–3089. (<https://doi.org/10.1016/j.ajpath.2015.07.004>)
- Russo J & Russo IH 1996 Experimentally induced mammary tumors in rats. *Breast Cancer Research and Treatment* **39** 7–20. (<https://doi.org/10.1007/BF01806074>)
- Schnitt SJ 2010 Classification and prognosis of invasive breast cancer: from morphology to molecular taxonomy. *Modern Pathology* **23** (Supplement 2) S60–S64. (<https://doi.org/10.1038/modpathol.2010.33>)
- Sflomos G, Dormoy V, Metsalu T, Jeitziner R, Battista L, Scabia V, Raffoul W, Delaloye JF, Treboux A, Fiche M, *et al.* 2016 A preclinical model for ERalpha-positive breast cancer points to the epithelial microenvironment as determinant of luminal phenotype and hormone response. *Cancer Cell* **29** 407–422. (<https://doi.org/10.1016/j.ccell.2016.02.002>)
- Shao Y, Sun X, He Y, Liu C & Liu H 2015 Elevated levels of serum tumor markers CEA and CA15-3 are prognostic parameters for different molecular subtypes of breast cancer. *PLoS ONE* **10** e0133830. (<https://doi.org/10.1371/journal.pone.0133830>)
- Sharma P & Allison JP 2015 The future of immune checkpoint therapy. *Science* **348** 56–61. (<https://doi.org/10.1126/science.aaa8172>)
- Siegel RL, Miller KD & Jemal A 2016 Cancer statistics, 2016. *CA: A Cancer Journal for Clinicians* **66** 7–30. (<https://doi.org/10.3322/caac.21332>)
- Sledge GW, Mamounas EP, Hortobagyi GN, Burstein HJ, Goodwin PJ & Wolff AC 2014 Past, present, and future challenges in breast cancer treatment. *Journal of Clinical Oncology* **32** 1979–1986. (<https://doi.org/10.1200/JCO.2014.55.4139>)
- Stampfer MR & Bartley JC 1985 Induction of transformation and continuous cell lines from normal human mammary epithelial cells after exposure to benzo[a]pyrene. *PNAS* **82** 2394–2398. (<https://doi.org/10.1073/pnas.82.8.2394>)
- Tekmal RR, Ramachandra N, Gubba S, Durgam VR, Mantione J, Toda K, Shizuta Y & Dillehay DL 1996 Overexpression of int-5/aromatase in mammary glands of transgenic mice results in the induction of hyperplasia and nuclear abnormalities. *Cancer Research* **56** 3180–3185.
- Tilli MT, Frech MS, Steed ME, Hruska KS, Johnson MD, Flaws JA & Furth PA 2003 Introduction of estrogen receptor-alpha into the tTA/TAg conditional mouse model precipitates the development of estrogen-responsive mammary adenocarcinoma. *American Journal of Pathology* **163** 1713–1719. ([https://doi.org/10.1016/S0002-9440\(10\)63529-8](https://doi.org/10.1016/S0002-9440(10)63529-8))
- Valdez KE, Fan F, Smith W, Allred DC, Medina D & Behbod F 2011 Human primary ductal carcinoma in situ (DCIS) subtype-specific pathology is preserved in a mouse intraductal (MIND) xenograft model. *Journal of Pathology* **225** 565–573. (<https://doi.org/10.1002/path.2969>)
- Van Keymeulen A, Lee MY, Ousset M, Brohee S, Rorive S, Girardi RR, Wuidart A, Bouvencourt G, Dubois C, Salmon I, *et al.* 2015 Reactivation of multipotency by oncogenic PIK3CA induces breast tumour heterogeneity. *Nature* **525** 119–123. (<https://doi.org/10.1038/nature14665>)
- Verbeke S, Richard E, Monceau E, Schmidt X, Rousseau B, Velasco V, Bernard D, Bonnefoi H, MacGrogan G & Iggo RD 2014 Humanization of the mouse mammary gland by replacement of the luminal layer with genetically engineered preneoplastic human cells. *Breast Cancer Research* **16** 504. (<https://doi.org/10.1186/s13058-014-0504-9>)
- Walsh NC, Kenney LL, Jangalwe S, Aryee KE, Greiner DL, Brehm MA & Shultz LD 2017 Humanized mouse models of clinical disease. *Annual*

- Review of Pathology* **12** 187–215. (<https://doi.org/10.1146/annurev-pathol-052016-100332>)
- Wang TC, Cardiff RD, Zukerberg L, Lees E, Arnold A & Schmidt EV 1994 Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice. *Nature* **369** 669–671. (<https://doi.org/10.1038/369669a0>)
- Watanabe Y, Takahashi T, Okajima A, Shiokawa M, Ishii N, Katano I, Ito R, Ito M, Minegishi M, Minegishi N, *et al.* 2009 The analysis of the functions of human B and T cells in humanized NOD/shi-scid/gammac(null) (NOG) mice (hu-HSC NOG mice). *International Immunology* **21** 843–858. (<https://doi.org/10.1093/intimm/dxp050>)
- Wege AK, Ernst W, Eckl J, Frankenberger B, Vollmann-Zwerenz A, Mannel DN, Ortmann O, Kroemer A & Brockhoff G 2011 Humanized tumor mice – a new model to study and manipulate the immune response in advanced cancer therapy. *International Journal of Cancer* **129** 2194–2206. (<https://doi.org/10.1002/ijc.26159>)
- Yanagawa M, Ikemot K, Kawauchi S, Furuya T, Yamamoto S, Oka M, Oga A, Nagashima Y & Sasaki K 2012 Luminal A and luminal B (HER2 negative) subtypes of breast cancer consist of a mixture of tumors with different genotype. *BMC Research Notes* **5** 376. (<https://doi.org/10.1186/1756-0500-5-376>)
- Zhang X, Podsypanina K, Huang S, Mohsin SK, Chamness GC, Hatsell S, Cowin P, Schiff R & Li Y 2005 Estrogen receptor positivity in mammary tumors of Wnt-1 transgenic mice is influenced by collaborating oncogenic mutations. *Oncogene* **24** 4220–4231. (<https://doi.org/10.1038/sj.onc.1208597>)
- Zhang X, Claerhout S, Prat A, Dobrolecki LE, Petrovic I, Lai Q, Landis MD, Wiechmann L, Schiff R, Giuliano M, *et al.* 2013a A renewable tissue resource of phenotypically stable, biologically and ethnically diverse, patient-derived human breast cancer xenograft models. *Cancer Research* **73** 4885–4897. (<https://doi.org/10.1158/0008-5472.CAN-12-4081>)
- Zhang XH, Giuliano M, Trivedi MV, Schiff R & Osborne CK 2013b Metastasis dormancy in estrogen receptor-positive breast cancer. *Clinical Cancer Research* **19** 6389–6397. (<https://doi.org/10.1158/1078-0432.CCR-13-0838>)
- Zhao H, Pearson EK, Brooks DC, Coon JST, Chen D, Demura M, Zhang M, Clevenger CV, Xu X, Veenstra TD, *et al.* 2012 A humanized pattern of aromatase expression is associated with mammary hyperplasia in mice. *Endocrinology* **153** 2701–2713. (<https://doi.org/10.1210/en.2011-1761>)

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