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

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 if 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 ERα 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 TGFα 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 Ca\(^{2+}\) 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, hydrenephrosis 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 mammoplasty (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/sun-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 mm\(^3\) 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-naive 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 TGFβ/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 micrometastases, 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).
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://brisken-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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