Universiteit
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The Netherlands

# Blueprints of disease: precision platforms for modelling breast cancer

Lutz, C.

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# CHAPTER 2

## General introduction: The complex landscape of luminal breast cancer

Catrin Lutz, Hendrik A Messal, Damir Vareslija[#] & Stefan Prekovic[#]

[#]shared senior authors

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## Abstract

The breast epithelium, vital for mammary gland function, is influenced by oestrogen through the oestrogen receptor (ER) signalling pathway. Luminal breast cancer (BC), characterised by ER expression, comprises the majority of all BCs, and presents significant clinical challenges due to therapy resistance and recurrence. Despite advancements in understanding luminal disease, improving long-term survival and reducing relapse of BC patients by predicting therapy efficacy and understanding resistance mechanisms remain critical challenges. This review discusses luminal BC biology, focusing on molecular classification of the primary disease, metastatic spread, and experimental models.

**Keywords**
breast cancer, steroid hormone receptors, metastasis, endocrine cancer, disease modelling

## Introduction

The epithelium found within the mammary gland is primarily involved in milk production during lactation. It comprises two main cell types: luminal epithelial cells, which line the ducts and are involved in milk production, and myoepithelial cells, which provide structural support and contract to facilitate milk release [1,2]. Steroidal hormones, oestrogens, play a key role in development, particularly in relation to female sexual characteristics, including the formation and function of the breast epithelium [3-6]. These cholesterol-based molecules can interact and thereby activate the Oestrogen Receptor (ER; refers to ERα), affecting the proliferation and differentiation of luminal epithelial cells [4]. By activating this Steroid Hormone Receptor (SHR), oestrogens contribute to ductal growth and branching during puberty, the menstrual cycle, and pregnancy [7,8].

Throughout the lifetime, alterations accumulated within cells of the mammary epithelium may alter the signalling pathways leading to the formation of breast cancer (BC) in 1 out of 8 women, despite preventative mechanisms [9]. Hormone receptor-positive (HR+) BC, characterised by expression of ER, holds particular significance due to its high prevalence (approx. 70% of all breast cancers) and the complexities it presents in clinical management [10]. Importantly, within the first 5 years of diagnosis, a hormone receptor-negative (HR-) subtype poses more risk of death, however, after that period the balance shifts with the majority of patients succumbing to HR+ disease [11]. While research efforts have deepened the understanding of HR+ disease, numerous clinical challenges persist. Particularly, long-term survival in BC patients is still hampered with many patients experiencing relapse of the disease, as we still lack definitive understanding of what cancer features predict therapy efficacy, recurrence, and which mechanisms control on-treatment progression. For instance, while many patients with HR+ BC initially respond well to endocrine therapies that disable the ER axis, a significant number of patients exhibit either intrinsic or acquired resistance [12]. Importantly, endocrine therapy resistance is a major cause of recurrence and often coincides with metastatic spread, significantly impacting patient outcomes.

Numerous of these challenges stem from the intrinsic heterogeneity that HR+ BCs present. In this review, we discuss the research advances in our understanding of HR+ BC biology. More specifically, we focus on the molecular diversity, the biology and patterns of metastatic spread observed in

HR+ BC, as well as contemporary experimental models used by the scientific community.

## Diversity and Biology of Primary HR+ BC Biology

### Molecular Classification of Luminal BC

Inherent diversity of HR+ BC is obvious even on a histopathological level. While most breast cancers originate from the ducts and invade the surrounding tissue (invasive ductal carcinoma (IDC); approximately 70% of all HR+ cases), a smaller portion arises from the lobules and has a diffuse growth pattern (invasive lobular carcinoma (ILC) 10-15% of all HR+ cases) [13]. In addition to these two prevalent forms, BC can also be mucinous (1-4%), cribriform (0.1-0.6%), or present as medullary, micropapillary, papillary or apocrine carcinoma [14,15]. Each of these histological subtypes has been linked to either favourable (e.g. IDC, mucinous, cribriform, medullary) or poor (e.g. ILC, micropapillary) long-term survival, however a high degree of variability exists within each of these groups [14]. This variability can, in a significant part, be explained by the molecular classification that arose from a pioneering effort to stratify breast cancers on the basis of transcriptional programs (**Figure 1**).

Toward the end of the last millennium, the Botstein group profiled gene expression of 42 different BC specimens using the then-emerging microarray technology [16]. By means of hierarchical clustering they identified four groups of samples with different molecular features of mammary epithelial biology: ER+/luminal-like, normal-like breast, basal-like and HER2+ subtypes. A further refinement based on analysis of 78 cancers, led to the discovery of two distinct subclasses of HR+ BCs (luminal A and B), characterised by differential long term outcomes, with luminal B tumours being more aggressive [17]. Particular genes involved in this stratification of BC became known as the PAM50 signature, a derivative of which has been approved by the FDA under the trademark name ProsignaTM, and may be utilised to assess a patient's risk of distant recurrence of disease, as well as benefit from adjuvant therapy [18,19]. A recent single-arm trial has shown that PAM50-derived ProsignaTM altered adjuvant treatment decisions across the risk groups of early BC patients, preventing unnecessary chemotherapy and reducing treatment decision discrepancies between hospitals. While the study demonstrates changes in treatment recommendations, it does not report any observed changes in survival outcomes at this stage, focusing primarily on improving

decision-making and patient quality of life [20]. Subsequent efforts led by the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) offered a detailed analysis of approximately 2000 BCs, providing further insight into the PAM50 subtypes [21]. On the basis of copy number profiling and gene expression analysis, ten integrative clusters were defined (**Figure 1**). Of particular interest is the Integrative cluster 2, found in very aggressive luminal A and B cancers linked to poor survival [21]. These cancers may be driven by genes found within the long arm of chromosome 11 (q13/14), including *CCND1* and *RSF1*. Further insights into HR+ BC came from The Cancer Genome Atlas (TCGA) initiative [22]. This revealed that luminal cancers generally express *ESR1, GATA3*, and *FOXA1* at high levels, and that the majority of these cancers have mutations in the PI3K pathway, including the *PIK3CA, MAP3K1*, and *MP2K4* genes (**Figure 1**). Additionally, *CCND1* amplifications were found in 58% luminal B and 29% luminal A cancers [22]. The TCGA, importantly, reported that luminal B cancers exhibit a higher degree of MYC and FOXM1 activity, as well as more *TP53* mutations in comparison to the luminal A counterparts [22]. In terms of biology, luminal A tumours are generally characterised by high expression of ER-target genes and low expression of proliferation-associated genes, explaining the better prognosis observed in initial studies [23]. Luminal B tumours, on the other hand, exhibit higher proliferation rates, leading to a more aggressive clinical course and may benefit from additional therapeutic interventions beyond endocrine therapy [24]. The aforementioned differences in ER-target and proliferation-associated gene expression, serve as markers to classify tumours into luminal A and B subtypes using immunohistochemistry (IHC). Particularly, ER+/HER- tumours that show Progesterone Receptor (PR) expression (>20% of all cells) with less than 20% of cells having Ki67 expressed (varying cutoffs used by different centres) are considered luminal A [25,26]. In contrast, luminal B cancers are characterised by low expression of PR, high expression of Ki67, and may potentially express HER2. Clinical categorization using IHC is, however, still difficult as a standardised scoring system does not exist [26,27]. Despite this, Ki67 status is often used to inform clinicians about prognosis, and even therapy response, of HR+ breast cancer patients. In addition to IHC and the above-mentioned ProsignaTM, various other expression-based signatures have been proposed and are used for prognostication of luminal BCs. Particularly important is MammaPrint, one of only a few FDA-cleared microarray assays available to date [28]. The MammaPrint prognostic signature consists of 70 genes, and may inform clinicians of the likelihood of postoperative recurrence/metastasis within 5 to 10 years. Initially, MammaPrint was designed only for patients with luminal

disease, however, nowadays it is used for any early detected HER2- cancer, regardless of ER status. While not FDA-approved, EndoPredict emerged as a very useful tool to aid clinical decision making due to its ability to estimate the risk of BC recurrence up to 15 years, as well as provide insight into whether individuals with early-stage HR+/HER- disease would benefit from chemotherapy [29,30]. Lastly, another commonly used gene expression assay is the Oncotype Dx® useful in providing a likelihood of recurrence within the first 10 years of diagnosis in individuals who will be treated with endocrine therapy for at least 5 years [31,32]. While Oncotype Dx® is generally accurate, studies have suggested that its accuracy may be lower in minority populations, implying that this issue may not be limited only to this gene expression assay [33,34]. While the classification of luminal BC works for the majority of cases, there are exceptions that further complicate the therapeutic decision making landscape. About 2-5% of all BCs present with low HR positivity (1-9%). These cancers have been suggested to resemble HR tumours on a molecular level, and generally have poorer survival outcomes compared to those with high receptor positivity [35,36]. Furthermore, HR+/HER2+ cases (±10% of all breast cancers) present a significant clinical challenge, as they are often associated with oestrogen independence, decreased sensitivity to chemotherapy, resistance to CDK4/6 inhibition, and, thus, poor outcomes. Therefore, for these cancers targeted therapies, such as EGFR/HER2 tyrosine kinase inhibitors, are considered and offered as a treatment strategy [37]. The molecular classification of luminal BC provides valuable insights into the disease's heterogeneity and has through the years had a positive impact on clinical decision making. Continued research, refinement, and clinical implementation of these classifications are essential for improving prognostic predictions and clinical outcomes for luminal BC patients.

## Steroid Hormone Receptors Shape Luminal Breast Cancer Biology

### General Aspects of Steroid Hormone Receptors

The biology, treatment response, and metastatic potential of luminal BC is tightly regulated by members of the SHR sub-family of nuclear receptors (**Figure 2**). Specifically, ER, PR, Androgen Receptor (AR), and Glucocorticoid Receptor (GR), play a pivotal role in the pathophysiology and management of luminal BC. These receptors act as ligand-activated transcription factors that regulate gene expression and are thus critical for cell proliferation,

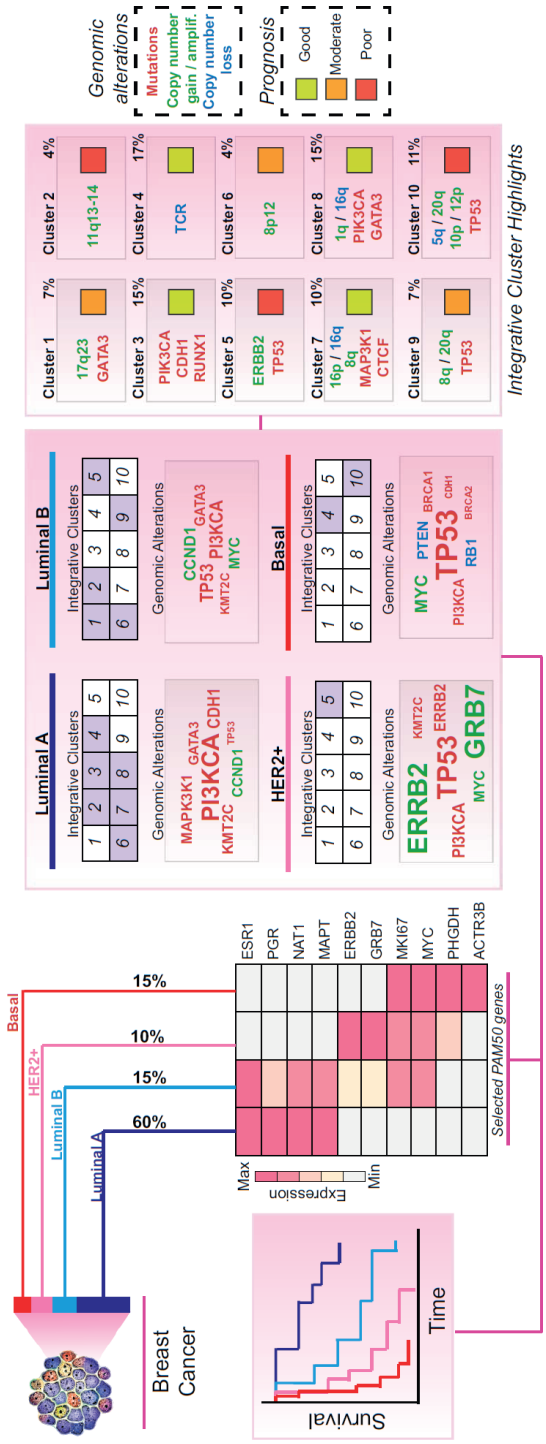


**Figure 1:** Molecular Classification of BC

BC is classified into distinct subtypes (Luminal A, Luminal B, HER2+, and Basal) based on the transcriptional profiles of genes in the PAM50 signature, each exhibiting unique survival characteristics. These subtypes can be further delineated into specific integrative clusters, characterised by particular genomic alterations such as mutations, copy number variations, and amplifications. Prognostic outcomes vary across these clusters, ranging from favourable to poor, providing critical insights into patient survival prospects.

differentiation, and survival, making them central to the development and progression of luminal BC [38].

In brief, SHRs have a modular structure, harbouring three distinct domains [39,40]. The amino-terminal domain, i.e. transactivation domain, is involved in co-regulator recruitment and has sites that can be post-translationally modified thereby affecting the receptor's function [40,41]. The central part of the SHR protein is represented by the DNA-binding domain with its zinc-finger functions responsible for direct interaction of SHRs with their DNA binding elements [39,40]. This domain is connected to the ligand-binding domain via a flexible hinge region that also may serve a function in fine-tuning receptor activity. The ligand binding domain contains a pocket that may structurally accommodate the respective ligand. Upon ligand binding, allosteric shifts in the protein occur, ultimately leading to activation of the receptor, engagement of DNA binding elements, recruitment of co-regulators and transcriptional modulation of their target genes [40]. In terms of their genomic action, the members of the SHR group predominantly bind enhancers, regulatory elements located far away from the promoters of target genes (**Figure 2**) [40,42-44]. Across the genome, SHRs bind thousands of enhancers that are brought in proximity to gene promoters by the 3D genome machinery to ultimately regulate the expression of hundreds of targets. Due to their role, as well as structure, the members of the SHR family are attractive drug targets, especially in the context of BC. Mapping of ligand binding pocket structures facilitated the design of various agonists and antagonists that have been in clinical use for decades, with new drugs still being developed and clinically tested. This makes SHRs, along with other nuclear receptors, a compelling proof-of-concept that transcription factor modulation, either activation or inhibition, can be successfully achieved in clinical settings, challenging the prevailing belief that targeting transcription factors therapeutically is unfeasible.

## Oestrogen and Progesterone Receptors

A crucial driver of luminal BC, ER, serves both as a therapeutic target and a response predictor to anti-oestrogen therapy [45]. Luminal BCs are typically treated with endocrine therapies that include direct antagonists, degraders, or selective oestrogen modulators, as well as aromatase inhibitors that block oestrogen signalling (an overview of current therapies has recently been published by [46]). In the adult mammary gland, ER action is essential for

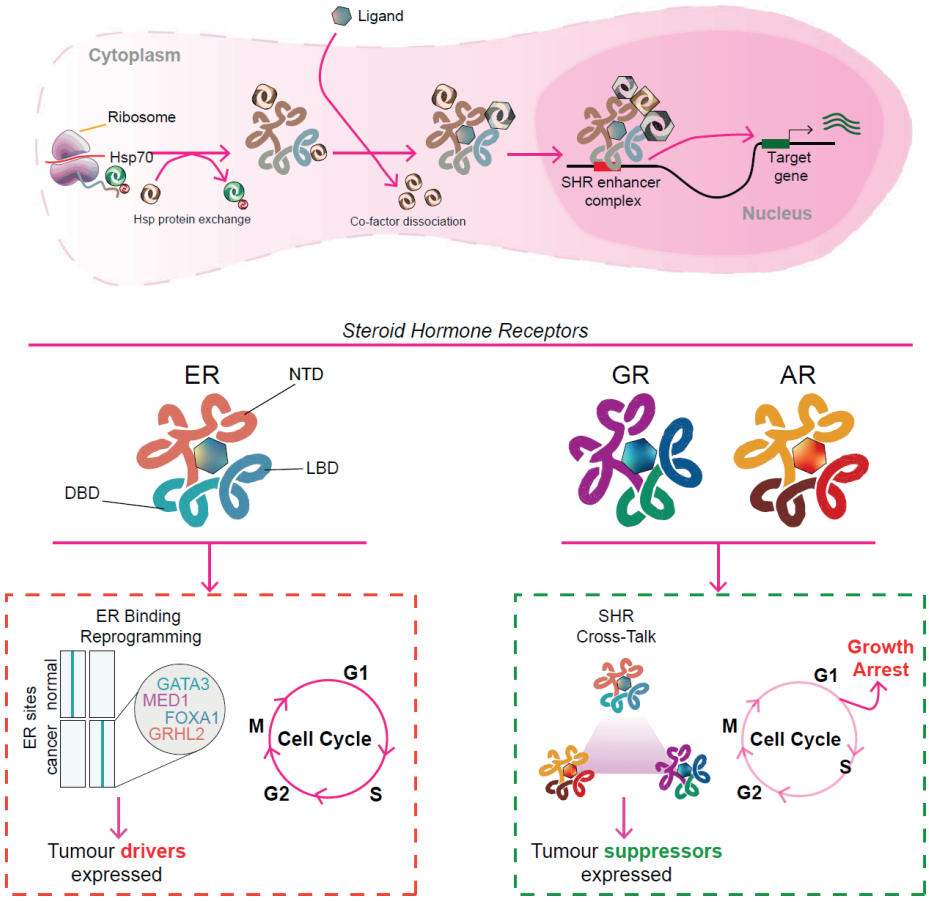


**Figure 2:** Steroid Hormone Receptors in BC

Illustration of steroid hormone receptor (SHR) signalling pathway. SHRs (including ER, GR, and AR) translocate to the nucleus upon ligand binding, where they bind to specific genomic elements within enhancers to ultimately regulate gene expression of hundreds of genes (top). In breast cancer ER binding is altered due to changes in co-interactors such as FOXA1 and GATA3, this drives a target gene program that supports cell cycle progression and proliferation. Conversely, GR and AR can engage in a cross-talk with ER to move it across the genome supporting a genetic program related to tumour suppression and thereby exit from the cell cycle.

differentiation, organisation and function of the epithelium, characterised by a distinct pattern of ER genomic engagement (**Figure 2**). In the process of tumourigenesis, it is suggested that GRHL2 can drive ER reprogramming and move it around the genome to drive a transcriptional program related to cancer-driving features [47].

In general, the interactions of ER with chromatin, and thereby its function, is facilitated by pioneer factors such as GATA3 and FOXA1 [48-50]. Alterations in binding of ER have been related to changes in both GATA3 and FOXA1 genomic interaction, suggesting that these pioneer factors are responsible for determining ER transcriptional programs [48-50]. This is not only true for GATA3 and FOXA1, as various other proteins have been shown to modulate ER binding events, including PR, GR, and AR, as further discussed below. Of particular interest is PR, as it is also an ER target gene, which was previously described to alter binding locations of ER, and its expression in BC to be related to better outcomes [51]. However, this is opposed by several other studies showing that PR action augments tumour proliferation and metastatic spread [52,53]. Furthermore, alterations of ER co-regulators (e.g. SRC-3 and MED1) may also modulate ER-driven transcription, thereby influencing tumour growth and therapy response. In addition to this, evidence suggests that genomic engagement, co-regulator recruitment, and transcriptional activity of ER can also be modulated by inputs from various signalling pathways such as PI3K/AKT and MAPK. Understanding the cross-talk of ER with other factors may open new avenues for therapeutic intervention and has been discussed elsewhere in detail [54]. Several studies have shown that patterns of ER engagement may relate to outcomes of luminal BC patients, especially in relation to endocrine therapy response [55,56]. These observations are supported by *in vitro* studies, as experiments in cell line models have suggested that resistance to ER-targeted therapies may also be mediated via changes in the ER genomic binding profiles and thereby target gene regulation [57,58]. Particularly interesting in terms of therapy resistance and disease progression, is the emergence of constitutively active ER mutants as well as fusions with other proteins, which seem to drive proliferation of BC, despite ER-inhibitor treatment and potentially drive alternative transcriptional programs that support metastatic cancer [59-61]. The above-discussed changes and versatility of ER action have been proposed to underpin insensitivity to current therapeutic approaches, urging us to better understand ER signalling and develop new ways to target the ER axis. One of the approaches that has emerged relies on combining multiple drugs that target several pathways simultaneously, suggesting this would effectively overcome endocrine resistance [62,63].

## Glucocorticoid Receptor (GR)

Mammary gland development and physiology is related to the action of the GR, with its expression ubiquitously reported across all cell types [64,65]. Expression of GR is diminished during cancerogenesis, implying it negatively impacts tumour development [66,67]. These implications have indeed been supported by various *in vitro* studies that show the negative effect of GR activation on cell cycle progression of luminal BC cell lines and xenograft models [68-70]. Numerous mechanisms have been proposed to underpin glucocorticoid-induced growth arrest across cancer models (the GR axis in cancer has been reviewed in detail by [71]), however in context of luminal BC the cross-talk of GR and ER is of particular interest and has been highlighted in numerous studies [70,72,73]. Of importance is a recent study that has identified GR to be crucial in determining luminal status of the disease [70]. Specifically, luminal A cancers have high GR activity, while luminal B cancers have low activity or even inactive GR signalling. The aforementioned cross-talk of GR and ER may underpin the growth suppressive features of responses to glucocorticoids. Specifically, upon treatment with glucocorticoids, both GR and ER were detected to occupy an enhancer element within an intron of the *ZBTB16* gene. This subsequent upregulation of *ZBTB16* may be a key to the diminished growth rates of BC once GR is activated. In terms of molecular classification, the observation that GR activity is, on average, higher in luminal A than B cancers may provide insight into further sub-stratification. Particularly, a predictive 8-gene classifier linked to GR activity has been derived from PAM50 [70]. This classifier allows identification of luminal A cancers that will have an unfavourable outcome and ultimately succumb to the disease. These lethal luminal A cancers are marked by loss of GR target-gene expression as well as loss of *CDH3, KRT17, KRT5, KRT14, EGFR, FOXC1, MIA*, and *SFRP1* expression. From the therapeutic standpoint, the majority of cancer patients are prescribed synthetic glucocorticoids to combat side effects related to chemotherapy. However, *in vitro* studies using BC cell lines suggest that adjuvant glucocorticoids may reduce chemotherapy effectiveness [74]. While clinical proof in BC is still missing, an open-label phase II study in ovarian cancer has indeed shown that inhibiting GR with Relacorilant augments chemotherapy response and improves survival outcomes in comparison to the monotherapy arm, warranting investigation in other cancer types [75,76].

## Androgen Receptor (AR)

Androgens have been reported to inhibit mammary cell proliferation, and affect ductal structures as well as the stromal compartments of the breast [77,78]. Expression of AR, which mediates the effects of androgens, has been observed in the majority of HR+ BC [79] . Tumours with expression of AR have, in general, been shown to be less aggressive and have been linked to favourable survival outcomes, while tumours that are AR- often present as larger and are of higher grade and thereby more often lead to death [79,80]. Similar to GR, it has been hypothesised that AR engages ER to modulate its action, therefore diminishing the proliferation-related signalling driven by ER. This is accompanied by upregulation of direct AR-target genes, including multiple genes related to tumour suppression [81]. Interestingly, AR action in BC has been shown to depend on GATA3, enabling it to control luminal epithelial cell differentiation [82]. In relation to AR activity, a transcriptional gene signature has been shown to be predictive of disease survival across multiple cohorts implying that it could be of use in patient stratification across luminal cancers, in a similar fashion as the ones related to GR activity [81].

The activation of AR to treat BC has emerged as a potential therapeutic concept, however natural or synthetic steroidal androgens often induce side effects [83]. Thereby a focus has shifted on selective AR modulators (SARMs) as they usually exhibit fewer side-effects, and have also shown promise in preclinical settings [81,82,84]. A clinical trial performed on 136 advanced HR+/HER2- BC patients has demonstrated that Enobosarm may have clinical benefit, strongly supporting future clinical studies in this setting [85]. Paradoxically, inhibition of AR has also been shown to have a therapeutic effect on HR+ BC progression [86-88]. This is particularly seen in the case of enzalutamide, a second generation anti-androgen. A recent study suggested that this intriguing finding is due to the direct inhibition of ER by enzalutamide, which is able to partially compete with oestrogens for binding to the ER pocket [88]. This was observed in BCs that express low levels of AR, but not the ones with high levels of AR. Conversely, activation of AR by SARMs led to inhibition of growth in models that expressed high levels of AR. These findings were supported by data from a cohort of 97 patients treated with endocrine therapy after metastatic recurrence, where it was observed that relative expression of AR and ER was predictive of outcome, and further suggesting supporting continuation of clinical investigation of AR agonism in BC [88].

## Other Nuclear Receptors

Beyond the classical steroid hormone receptors, several other nuclear receptors significantly influence HR+ disease through both ER-dependent and ER-independent mechanisms. Particularly, there evidence is emerging that peroxisome proliferator-activated receptor gamma (PPARγ), retinoic acid receptors (RARs), vitamin D receptor (VDR), liver X receptors (LXRs), farnesoid X receptor (FXR), and oestrogen-related receptors (ERRs) affect HR+ BC cells, changing their growth and/or affecting therapy response. Particularly interesting are nuclear receptors that control cellular metabolism and differentiation. Each regulating a different facet of lipid-associated metabolism, LXRs, FXR and PPARγ, have all been shown to engage in a crosstalk with the ER, but also affect BC cells via ER-independent pathways [89-92]. While activation of these receptors has been related to growth inhibition, there is some evidence that they also may influence effectiveness of hormonal treatments [93,94]. The latter, however, should be the focus of future studies. Furthermore, nuclear receptors involved in cell proliferation and differentiation may also significantly influence BC biology. The interaction between ER and RARs, which mediate the effects of retinoic acid, modulates cell proliferation and can enhance the efficacy of anti-oestrogen therapies, while also exerting independent effects on gene expression related to growth and differentiation [95,96]. Similarly, activation of the VDR by vitamin D reduces ESR1 gene expression, affecting ER signalling and enhancing tumour growth inhibition through both ER-dependent and independent mechanisms [97]. Research efforts around ERRs (a group of nuclear receptors that share structural similarities with ER but are unable to bind oestrogen) suggest that these may influence BC progression [98,99]. By engaging in crosstalk and thereby modulating ER signalling, ERRs may affect ER-related target gene expression [100]. In particular, ERRα, has been implicated in disease progression and may contribute to resistance to endocrine therapies [101], while also independently regulating energy metabolism and mitochondrial function [102].

Collectively, nuclear receptors beyond the SHR family may impact cell proliferation, apoptosis, and differentiation through multiple pathways, highlighting their potential as additional therapeutic targets to improve BC treatment.

## Luminal Breast Cancer Metastasis

### Emergence of Luminal Breast Cancer Metastasis

Although the majority of luminal cancers are initially sensitive to therapy, resistance often develops due to genetic and epigenetic alterations leading to disease progression and ultimately metastatic spread. Metastatic relapse in BC can occur regardless of the primary tumour's clinical and molecular characteristics. As highlighted above, patients with ER+ BC initially have a better prognosis, but they remain at significant risk for late recurrence and may present with metastatic disease decades later [103,104]. Studies have identified four late-recurring integrative subtypes in luminal tumours, which are associated with high risks of recurrence up to 20 years post-diagnosis [103]. The extended latency period in luminal tumours is thought to be due to their ability to survive in a quiescent, therapy-resistant state until they re-enter proliferation and form detectable metastatic lesions (**Figure 3**).

Metastasis is a dynamic process in which cancer cells acquire specialised traits that enable them to evade the immune system, resist cell death, and establish growths in distant organs. This process involves several steps: migration from the primary tumour, engagement with the stromal environment, penetration of blood vessels, and adaptation to new microenvironments (**Figure 3**) [105]. The initial step of the metastatic cascade involves cancer cells breaching the extracellular matrix (ECM) and migrating. Specifically, BC can disseminate via the lymphatic system, with lymph node metastasis being a crucial parameter in BC staging [106]. In addition, BC cells can escape the primary tumour site and enter the bloodstream as Circulating Tumour Cells (CTCs), either singly or in clusters [107]. CTC clusters exhibit genome methylation patterns indicative of a stem-like state, characteristic of metastasis-initiating cells [108]. A small proportion of CTCs in BC patients are bound to neutrophils, which enhances their proliferative and metastatic potential [109,110]. Patients with CTC-neutrophil clusters exhibit faster disease progression compared to those without such clusters [109,110]. Importantly, CTCs can home to secondary organs as Disseminated Tumour Cells (DTCs) and eventually form macrometastases (**Figure 3**).

### ER+ dormancy

Circulating cells that extravasate at the target site face harsh conditions that challenge their survival and notably most cancer cells that manage to extravasate do not lead to metastases [111,112]. Instead, these cells often remain

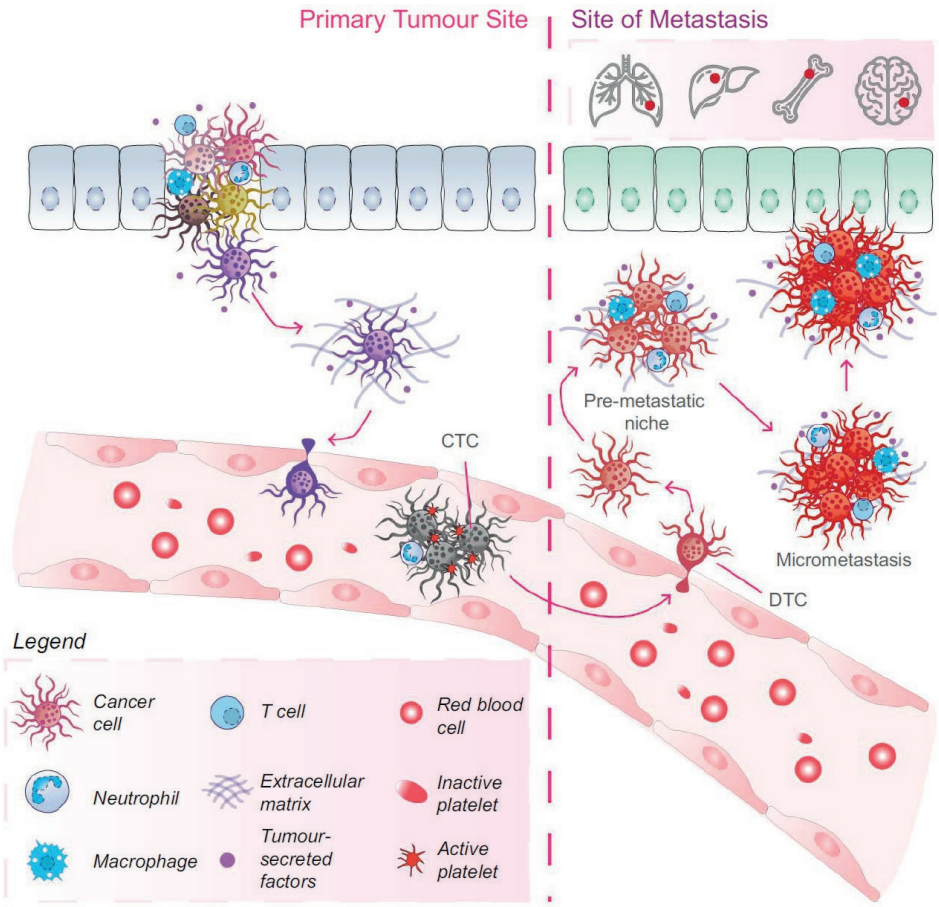


**Figure 3:** BC Metastasis

Schematic representation of the metastatic cascade and tumour microenvironment in cancer progression. The figure details the journey of cancer cells from the primary tumour to distant sites, including the formation of pre-metastatic niches and micrometastases. Key cellular components, such as cancer cells, immune cells (neutrophils, macrophages, T cells), extracellular matrix, tumour-secreted factors, red blood cells, platelets (inactive and active), circulating tumour cells (CTCs), and disseminated tumour cells (DTCs), are illustrated, showing their roles and interactions within the tumour microenvironment.

isolated or form small clusters called micrometastases, entering a state of dormancy [111,112]. Dormancy in cancer cells can be maintained through several mechanisms: (1: cellular dormancy) the absence of growth factor signalling and the presence of metastatic suppressor genes, (2: angiogenic dormancy) the lack of an activated angiogenic switch, and (3: immunologic dormancy) the influence of immunological factors [113]. During this dormant

phase, cancer cells adapt to their environment by downregulating certain proteins, enabling them to survive in a quiescent state with reduced reliance on typical growth and survival pathways. In patients with HR+ BC, dormant cells pose a significant challenge as they can evade treatment and persist for extended periods. Various studies have elucidated mechanisms and factors contributing to dormancy in HR+ disease. For instance, an *in vitro* evolutionary study demonstrated that endocrine therapies induce non-genetic cell state transitions into dormancy in a stochastic subset of cells via epigenetic reprogramming [114]. Functional genomics has revealed that cis-regulatory elements play context-dependent roles in dormancy and endocrine therapy resistance, with their perturbation showing specific impacts on these processes [115]. Profiling the epigenome of luminal BC specimens has identified key regulatory elements, such as the transcription factor YY1, which regulates ER activity and contributes to endocrine treatment resistance, underscoring the role of epigenetic mechanisms in phenotypic heterogeneity [116]. Additionally, signalling through the leukaemia inhibitory factor receptor (LIFR) has been associated with inducing dormancy in luminal BC cells [116]. Metabolic adaptations are also crucial for dormant ER+ BC cells, which evade anti-oestrogen therapies by upregulating fatty acid oxidation (FAO) and activating the AMPK pathway. Hypoxia further allows these cells to resist ER-targeting therapies through ERK activation, and inhibition of FAO and ERK improves the efficacy of anti-oestrogen drugs [117,118]. The microvascular niche protects dormant cells from chemotherapy via integrin-initiated signalling, although inhibition of integrins β1 and αvβ3 can sensitise these cells to treatment [119,120]. Additionally, angiopoietin 2 (ANGPT2) is linked to hormone therapy resistance in dormant cells, with bone marrow endothelial niches inducing dormancy that can be reversed by ANGPT2 signalling [121]. Tamoxifen and fulvestrant induce dormancy by reducing oxidative phosphorylation (OXPHOS), potentially by affecting complex I of the respiratory chain [122]. Some cells, although, may exit dormancy through mitochondrial DNA transfer via extracellular vesicles, restoring OXPHOS and proliferation [123]. The kinase p38α–MSK1 axis has been identified as a regulator of metastatic dormancy, with low MSK1 expression correlating with early metastasis. Particularly, downregulation of MSK1 enhances bone homing and growth capacities by controlling genes required for luminal cell differentiation [124]. The p38 mitogen-activated protein kinase (MAPK) pathway plays a crucial role in maintaining tumour cell dormancy in breast cancer. Activation of p38 signalling induces a quiescent state in DTCs, allowing them to survive in distant organs like the bone marrow for extended periods. This dormancy contributes to endocrine

resistance, as dormant cells are less susceptible to therapies targeting proliferating cells. It has been demonstrated that sustained p38 activation leads to growth arrest in carcinoma cells, highlighting its role in dormancy regulation [125,126]. Furthermore, targeting the p38 pathway has been shown to awaken dormant cells, potentially sensitising them to chemotherapy [126]. In addition, amplification of MAF in luminal BC promotes a chromatin landscape favouring metastasis, with KDM1A playing a key role in this epigenomic reprogramming. Loss of KDM1A activity prevents metastasis, indicating the interplay of genetic, epigenetic, and hormonal signals in metastasis [127]. These studies collectively highlight the complex and multifaceted nature of dormancy in luminal BC, emphasising the importance of targeting dormant cells to prevent disease recurrence and improve patient outcomes.

## Genetic Profile of Metastatic ER+ BC Cells

The genetic landscape of metastatic HR+ BC cells reveals a range of molecular alterations associated with their metastatic capability and resistance to therapy. Receptor expression changes during disease progression, with ER+ to ER- conversion occurring in approximately 10-20% of cases, and gene expression subtype discordance occurring even more frequently [128-134]. Loss of ER expression at relapse is linked to driver mutations in *TP53* and *ARID1A*, with *ARID1A* mutations being more common in relapse and associated with HR loss [132]. *ARID1A* and other SWI/SNF complex components appear crucial for responding to ER antagonists, with *ARID1A* deletion resulting in endocrine resistance and increased sensitivity to bromodomain inhibition [135]. Through the analysis of genomic and clinical data across 50 tumour types, significant correlations between chromosomal instability and metastatic burden have been identified, notably in ER+ BC. Specifically, in ER+/HER2− BC, *TP53* mutations show a strong correlation with metastatic burden, highlighting its critical role in guarding against chromosomal instability [136]. Additionally, other studies highlight the significance of chromosomal instability and specific genetic alterations, such as *CBFB* mutations, in determining metastatic patterns and burden [136]. Other genomic analyses of metastatic lesions reveal modestly increased frequencies of known pathogenic variants like *PTEN, RB1*, and altered mutational signatures [136,137]. *ESR1, PIK3CA*, and *GATA3* mutations are more frequent in HR+ metastatic BC, with *PIK3CA* mutations particularly prevalent in luminal BC that have lost ER expression [138]. Other studies identified nine driver genes more frequently mutated in ER+/HER2− metastatic BC, with *ESR1* mutations enriched in liver metastases [139] and generally enriched in metastatic sites regardless of ductal or lobular status [136]. *ESR1* mutations,

particularly in the ligand-binding domain, appear to be significant drivers of therapeutic resistance, appearing in 20-40% of previously treated metastatic tumours. These mutations are predominantly acquired under oestrogen deprivation, enabling oestrogen-independent expression of ER target genes [140-144]. Structural variants of *ESR1*, including fusions with YAP1 or PCDH11X, also contribute to resistance but can be countered by CDK4/6 inhibitors [145,146]. Additionally, functional studies have shown that *CYP19A1* amplification, causing increased aromatase activity, arises in aromatase inhibitor-resistant metastatic patients, promoting local autocrine oestrogen signalling [147].

Alterations in the growth factor pathways including MAPK, ERBB2 and NF1, are also enriched in endocrine-resistant tumours. These alterations often predict limited benefit from endocrine therapies and suggest alternative therapeutic targets, such as *ERBB2*-activating mutations and NF1 loss [148,149]. Additionally, the FGFR4 pathway has been implicated in subtype switching from luminal A to HER2-enriched metastases. Inhibition of FGFR4 signalling reversed this switch, suggesting its role in metastasis and therapeutic resistance [150].

### Interaction of Luminal BC Cells with the Metastatic Environment

Luminal BC cells exhibit distinct interactions with their metastatic environments, which influence dormancy and outgrowth dynamics across different organs. Notably, the bone is the most frequent site of metastatic spread for luminal BC, and this preference may be due to several factors. Firstly, breast cancer cells may thrive in environments sharing similar molecular properties to their tissue of origin; for instance, the prominence of RANK/RANKL signalling, vitamin D, and RUNX2 expression in bone tissue creates a conducive environment for these cells [151]. Secondly, the bone microenvironment could influence the accumulation of *ESR1* mutations, potentially fostering endocrine resistance; however, current evidence is insufficient to confirm a specific role of these mutations in bone metastasis. Moreover, while DTCs often lose ER expression [152], most overt bone metastases remain ER+ and respond to endocrine therapies, although resistance frequently develops [153]. This paradox suggests that the bone microenvironment may confer endocrine resistance, particularly through interactions with osteogenic cells, implying that additional mechanisms are involved [154]. Furthermore, the bone marrow microenvironment has been suggested to significantly contribute to the dormancy of breast cancer cells. Mesenchymal stem cells (NG2+/Nestin+) in the bone marrow produce TGFβ2 and BMP7, which activate quiescence pathways in DTCs, supporting their dormancy [155]. The interaction between

cancer cells and their microenvironment during invasion and metastasis involves complex signalling pathways, including calcium flux, which has been implicated in the progression of luminal BC to bone metastasis [156]. Bone marrow biopsies can identify DTCs in early-stage BC patients, aiding in the detection of microscopic disease [157,158]. The osteogenic niche transiently reduces ER expression in bone micrometastases, leading to endocrine resistance mediated by EZH2, which drives these cells towards a basal and stem-like state [159,160]. Although no FDA-approved therapies specifically target dormant cells, clinical trials are exploring autophagy inhibitors, CDK4/6 inhibitors, and checkpoint inhibitors to eradicate dormant cells in the bone marrow (www.clinicatrials.gov; NCT04841148, NCT04523857, NCT03032406).

On the other hand, the delayed development of brain metastasis in HR+ BC suggests that luminal tumour cells may uniquely adapt to the brain environment, unlike ER- cells, which metastasise faster and have shorter survival times after brain metastasis. Recent findings indicate that luminal tumour cells undergo extensive transcriptional remodelling, which may provide them with intrinsic characteristics that enhance their ability to metastasize to the brain [131]. Charting the m6A-methylome has revealed global methylation changes in ER+ metastasis, with FTO inhibition reducing tumour growth, offering new therapeutic strategies [161]. Metastatic brain cancers of luminal origin often exhibit a mutational signature associated with homologous recombination deficiency [131]. In these metastases, frequent *TP53* mutations and *NF1* deletions are observed, which are linked to endocrine resistance and tumour progression [131]. Recently, RET (a known ER target gene) has been implicated in controlling the colonisation and expansion of BC brain metastases [162,163]. Additionally, targeting ADAM22 and ER co-regulators AIB1 and SRC1, which regulate pro-metastatic pathways, showed promise in managing HR+ brain metastasis [164-166]. The immune compartment also significantly regulates the balance between DTC dormancy and metastatic outgrowth. Sustained lung inflammation, caused by factors such as tobacco smoke or lipopolysaccharide exposure, can convert dormant cancer cells into aggressively growing metastases by inducing neutrophil extracellular traps (NETs) [167]. NETs remodel the extracellular matrix through proteases like neutrophil elastase and matrix metalloproteinase 9, which activate integrin α3β1 signalling and induce proliferation of dormant cancer cells [167].

The aged stroma, including immune cells, fibroblasts, and extracellular matrix components, plays a crucial role in promoting tumourigenesis. Older patients

are more likely to experience late-onset relapse, with 80% of recurrences at ≥10 years post-diagnosis occurring in patients older than 60 [168,169]. Studies using mouse models of luminal BC have shown that DTCs display a dormant phenotype in young mice but exhibit accelerated metastatic outgrowth in aged or fibrotic microenvironments. The aged microenvironment promotes DTC proliferation through PDGF-C signalling, which can be blocked by PDGFRα inhibitors or PDGF-C-blocking antibodies [170]. Collectively, these studies highlight the intricate interactions between HR+ BC cells and their metastatic environments, emphasising the need for targeted therapies that address the specific mechanisms driving dormancy and metastatic outgrowth.

## Disease Modelling for Luminal Breast Cancer

### Cell line models

Since the establishment of BT-20 cells in 1958 , and after overcoming technical difficulties in extracting viable tumour cells and their long-term propagation, a growing number of BC cell lines have been generated (**Figure 4**) [171-173]. Cell line models are appealing as they provide an unlimited, homogeneous, and cost-effective material source, making them excellent models for studying various aspects of BC tumour biology (**Figure 4**) [174,175]. To date 115 human BC cell lines are described in the Cellosaurus database, 92 are available in the DepMap portal (Broad Institute), 74 are classified in Cell Model Passports (Wellcome Sanger Institute) and 69 lines are genomically analysed in the COSMIC project (Wellcome Sanger Institute). These efforts alongside large-scale genomic characterisation projects of cancer cell lines lead to the establishment of large annotated cell line collections (**Supporting Table 1**) enabling preclinical breakthroughs such as the identification of lineage, genetic, and gene-expression-based markers of drug sensitivity retro- and prospectively [176,177].

Luminal BC cells, like luminal BC patients, are characterised by ER and/or PR expression, as well as gene or protein markers indicative of luminal features (e.g., luminal keratins KRT8/18/19, and transcription factors such as GATA3 and FOXA1). Despite over 75% of BC patients presenting with HR+ disease, only 21% (19/92) of characterised BC cell lines are classified as luminal, with no distinction made between luminal A or luminal B subsets. Additionally, 14% (13/92) are annotated as HER2+ based on HER2 amplifications. This strong bias towards the establishment of ER- cell lines was observed early on. Ethier

hypothesised that extracellular matrix proteins and an autocrine feedback loop, supported by secreted growth factors, facilitate cell adherence and growth of ER- cells *in vitro* [178]. Moreover, luminal cell lines are generally more differentiated with reduced migratory propensity, which is suggested to be due to tight cell-cell junctions consistently observed at the tumour level as well [179].

Cell lines, despite their extensive usability and utility, present significant limitations beyond their misrepresentation of clinical subtype incidence. Many BC cell lines, especially those of luminal identity, are derived from metastatic sites rather than primary tumours. In fact, 41% (38/92) of all characterised cell lines and only 16% (3/19) of established luminal cell lines originate from a primary breast tumour. In contrast, 57% (52/92) of all lines and 84% (16/19) of luminal lines, including the widely used MCF-7 line, stem from metastatic sites and pleural effusions. This introduces a potential bias toward more aggressive cells with high invasion potential, compared to primary tumours. Moreover, cells are known to accumulate mutations during initial establishment and subsequent cultivation, further introducing a bias toward more aggressive disease culturing *in vitro*. Alterations in cell lines are approximately twice as frequent compared to tumours [180,181]. This may, for example, explain the variable sensitivities to tamoxifen observed in MCF-7 cell lines and is reflected in the different categorizations of cell lines in literature [182]. Luminal cells in particular seem prone to developing distinct populations under different culture conditions, as evidenced by differences reported in ER, PR, or HER2 status [171,180,183-185]. Additionally, cell line assays lack the tumour microenvironmental factors shown to influence cell signalling and alter cell molecular features. For instance, fibroblasts have been shown to stimulate the expression of luminal keratins in basal cells and basal keratins in luminal cells [186]. Similarly, culture conditions with high or low EGFR activity can render previously ER+ cells ER- and vice versa [186,187]. Most studies, including the aforementioned comprehensive cell encyclopaedic initiatives, do not further distinguish between luminal cell subtypes. Stratification into luminal A and B cell lines would improve tumour modelling and specifically enhance the outcomes of drug response studies that depend on ER/HER2 status or cell proliferation. This distinction is particularly vital as luminal B cells, characterised by a higher proliferation index, are more aggressive and invasive than luminal A types. Comprehensive characterisation and library creation, as described by Barretina et al. and Garnett et al., along with the efforts of projects like the Broad Institute's DepMap cell line collection and the Sanger Institute's Cell

Model Passports and COSMIC database, are crucial [176,177]. These initiatives aim to achieve better molecular subtyping and classification of existing cell lines, thereby enabling more informed use in future exploratory and preclinical studies.

### Primary and Patient-derived xenograft organoids (PDXOs)

Although previously mentioned *in vitro* models have contributed tremendously to BC research, they also harbour various drawbacks. Overcoming limitations of cell lines as prevalent modelling platforms, three-dimensional organoid technology gained popularity over the past decade, offering an elegant solution to model organ development as well as various pathologies in a dish whilst maintaining some of the complexity of spatial growth metrics (**Figure 4**) [188]. Mammary gland organoids were lacking behind gastrointestinal, reproductive tract, and lung tissues until Linnemann et al. described the first freshly isolated human mammary epithelial cell cultures, maintained in floating collagen gels and resembling TDLUs astonishingly closely [189]. Within the following three years, tissues from over 150 women with BC resulted in the generation of a large living biobank encompassing more than 100 BC organoid lines and the establishment of their culture conditions [190]. Remarkably, the established organoids resemble histologic and genetic features of the primary tumours they were sampled from and successfully mirrored the diagnostic BC spectrum observed in clinical practice. The lasting hurdle of maintaining these mammary organoids in culture long-term could be overcome by further improvement of their culture conditions, enabling culture expansion and with this the opportunity for large-scale manipulation, screening and xenografting studies, rendering organoid technologies an important new tool in BC research [191]. In addition to primary human organoids, recent work by Guillen *et al.* reports 40 established Patient-Derived Xenograft Organoid (PDXO) lines with various clinical parameters, including 13 ER+ lines amongst which 11 also stained positive for PR [192]. Interestingly, the authors were able to test 16 different culturing conditions and established that PDXO growth was mainly supported by a Rho kinase inhibitor (Y-27532) with the addition of N-acetylcysteine (NAC) for ER+ organoid culturing, allowing for simplified medium composition from the one previously described by Sachs *et al.* and used for primary organoid culturing. Established ER+ lines only insignificantly downregulated ER and its target genes over several culture passages. Upon re-engraftment, outgrown tumours responded to fulvestrant treatment indicating that even long-term ER+ PDXO cultures could maintain functional ER signalling whilst retaining endocrine therapy sensitivity.

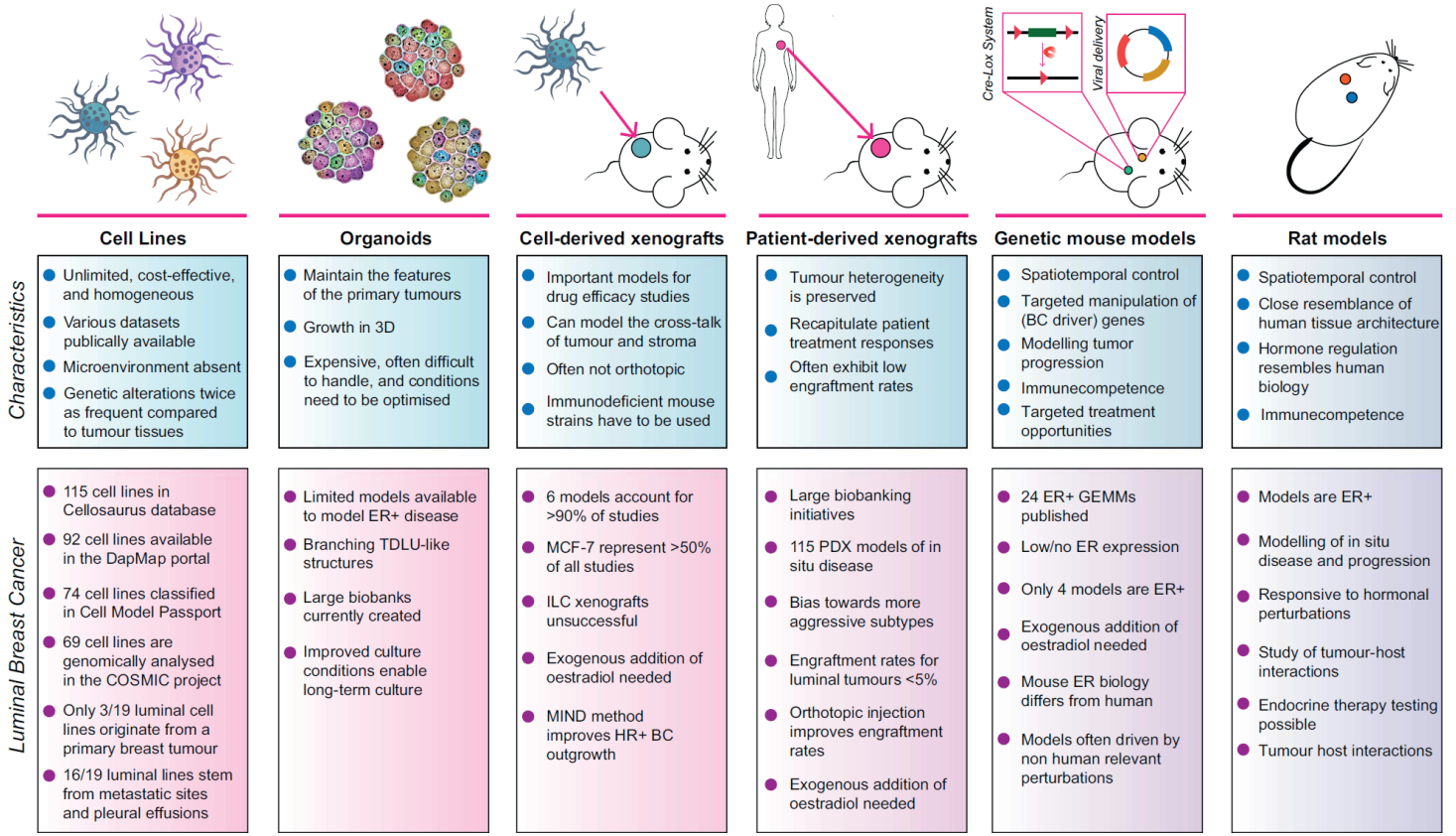


**Figure 4:** Experimental Models in BC

Comparison of experimental models used in breast cancer research. The overview of cell lines, organoids, xenografts models, genetic mouse models, rat models. Each model's characteristics are discussed. The figure emphasises the advantages and limitations of each model in studying luminal breast cancer.

## Cell line-derived xenograft (CDX) models

Interpreting cell line-derived data within the context of a complex microenvironment, which encompasses intricate signalling cascades and the interplay of various cell types and lineages, adds a crucial dimension to cancer research. To achieve this, BC cell lines are often transplanted into immunodeficient mice, resulting in Cell line-Derived Xenograft (CDX) models that remain valuable assets in preclinical BC research (**Figure 4**) [175,179,193]. These human cell line xenografts provide opportunities to study tumour-host interactions in fields such as endocrinology, immunology, and tumour-stroma cross-talk and are predominantly used in preclinical drug efficacy studies. Humanised mouse models, which involve immunodeficient mice co-engrafted with human tumour cell lines and innate immune populations, have emerged as a promising tool to further enhance tumour-host interaction studies [194]. Despite the availability of numerous published BC cell lines, most studies rely on a limited set of models that fail to capture the heterogeneity of human BC. Specifically, only six models (MCF-7, MDA-MB-231, T-47D, SK-BR-3, MCF10A, and MDA-MB-468) account for over 90% of studies employing BC cell lines, with MCF-7 alone representing more than half of all studies [195]. Moreover, they require supplementation with oestradiol, resulting in non-physiologically high serum oestradiol levels [195-198]. Furthermore, most CDX modelling studies rely on systemic delivery approaches, subcutaneous injection, or the transplantation of cells into the mammary fat pad of immune-compromised mice. These approaches tend to introduce a bias towards the outgrowth of predominantly ER- cell clones and result in tumours that grow much faster than their human counterparts [199,200]. Injecting tumour cells into adipose tissue does not accurately mimic human BC, which originates from the Terminal Ductal Lobular Units (TDLUs) of mammary ductal epithelial cells [201]. Additionally, these approaches tend to model late-stage aggressive invasive disease, selecting for metastatic cells rather than representing initial and primary breast carcinoma [202].

To overcome these limitations, the Mouse Intraductal (MIND) model was developed, which involves injecting tumour cells directly through the nipple into the mammary gland ductal system [203]. Initially used to study Ductal Carcinoma *in situ* (DCIS), this model has proven effective in orthotopically mimicking early disease onset. The MIND model has since demonstrated its ability to recapitulate various stages of BC and successfully maintain cell line characteristics after xenografting *in vivo*. Comparative analysis between non-orthotopic and MIND-transplanted breast cancer tumour cell outgrowths

highlights the importance of the tissue microenvironment, particularly for the engraftment and growth of luminal BC cells [200,204]. Intraductal xenografts allow tumour cells to grow in the presence of physiological hormone levels, closely resembling human conditions, and resulting in better preservation of luminal cell characteristics, including HR status [200].

### Patient-derived xenograft (PDX) models

Patient-Derived tumour Xenograft (PDX) models are especially appealing due to the preserved tumour heterogeneity and patient tumour features in an *in vivo* modelling system (**Figure 4**) [205]. Although PDX models are propagated through successive *in vivo* passaging in mice, molecular changes that occur in long-term *in vitro* culture are mostly avoided and tumour intrinsic features remain present over numerous *in vivo* passages [199,202]. As these models exhibit similar responses to treatment as observed in the clinic, significant efforts have been made to create a well-characterised collection of breast cancer PDX models covering various clinical subtypes and molecular features [192,206,207].

However, mouse transplantation models have several limitations. They often exhibit low engraftment rates, particularly for luminal BC subtypes, and tend to favour more aggressive, HR- disease subtypes. Additionally, these models frequently require supplemental oestradiol, as endogenous mouse oestrogen levels are typically inadequate to support xenograft growth [208]. Currently, most transplantation models represent HR- BC, with only a limited number available for HR+ BC. Even fewer models exist for the luminal A subtype, which is the most prevalent in clinical settings [209]. In fact, luminal tumour engraftment rates are typically reported to be below 5%, compared to over 25% for HR- counterparts [210]. This disparity is particularly pronounced for lower-grade, less aggressive, treatment-naïve luminal A tumours [199,202]. Similarly, ductal and lobular carcinomas *in situ*, despite their increasing incidence, are underrepresented in current PDX models [211]. These ER+ non-obligatory precursors to human luminal tumours still lack reliable prognostic biomarkers to determine when treatment is necessary to prevent progression to invasive luminal breast cancers. Non-orthotopic transplantation techniques, where cells are injected subcutaneously or xenografted into the mammary fat pad, as used in CDX and most PDX models, exhibit higher expression of basal markers as well as EMT and TGFβ/SLUG signalling [200,204]. This research suggested that the fat pad microenvironment therefore induces xenografted luminal tumour cells to undergo basal differentiation, inhibiting the growth of luminal cells and potentially explaining the engraftment bias toward HR-

tumours or the loss of ER expression [200,203,211]. However, this TGFβ-induced basal cell differentiation appears to be circumvented in MIND models, where it is suppressed by the intraductal environment, which has shown significantly improved engraftment rates for HR+ patient tumour samples (average of 74%) whilst maintaining hormone receptor expression [200,211]. Leveraging the MIND technology to develop models for *in situ* stages of luminal breast disease, a biobank of DCIS-PDX mouse models was recently established. This biobank characterised 115 patient-derived mouse-intraductal DCIS models, including two novel distributable luminal A and four luminal B DCIS models [212].

## Genetically and somatically engineered mouse models (GEMMS & SEMMs)

Studies in the aforementioned models have yielded significant insights into the pathogenesis and therapy response of luminal BC [213,214]. Despite these advances, the inherent limitations of these models in fully capturing immune-microenvironmental interactions necessitate alternative approaches. Cancer, being fundamentally a genetic disorder, can be more effectively studied using Genetically Engineered Mouse Models (GEMMs) (**Figure 4**). Mice are advantageous for this purpose due to their ease of breeding, relatively short generation times, homogeneous genetic background, and amenability to genetic manipulation. The exploration of cancer biology is enhanced by using GEMMs, which allow for detailed investigations of immune responses to tumourigenesis and enable targeted manipulation of genes involved in specific cancer types. To date, 24 different GEMMs for modelling of ER+ BC have been published, ranging in their strain background, lesion penetrance, tumour latency, and ER positivity. These models were previously neatly reviewed [215-217] and can be classified into 6 different categories: (1) Models with direct overexpression of ER in mammary epithelium cells, (2) Genetic perturbation of oestrogen signalling pathway members, (3) Genetic perturbations in non-canonical oestrogen related pathways, (4) Pharmacologically-induced tumours in models harbouring genetic alterations impacting oestrogen signalling, (5) Chemically-induced mammary tumour models in animals with genetic perturbations altering oestrogen signalling, and (6) Spontaneously developing ER+ mammary tumours resulting from sibling matings in nude mice.

Models from the first and second categories predominantly employ the mouse mammary tumour virus (MMTV) promoter to overexpress ER or viral oncoproteins such as the polyomavirus middle T (PyMT) antigen in mouse

mammary epithelium [218,219]. However, these models frequently develop tumours that either lack ER expression or lose ER expression at later stages [218,220]. Furthermore, GEMMs developed through genetic alterations in oestrogen signalling molecules or oncogenes, relay on the rat neu-related lipocalin (Nrl) or Whey Acidic Protein (WAP) promoters, primarily produce mammary tumours that are HR− or lose ER expression under endocrine therapy [221-223].

To date, only four GEMMs have been reported to develop lasting HR+ mammary tumours: the *Stat1*-knockout, *BLG-Cre;Kras*$^{G12V}$, *NRL-PRL*, and *Wap-Cre;Pik3ca*$^{H1047R}$ [224-227]. While the *Stat1*-knockout, *BLG-Cre;Kras*$^{G12V}$, and *NRL-PRL* models produce HR+ mammary tumours that respond to hormonal changes such as ovariectomy and anti-oestrogen treatment, they are constrained by their driver mutations [225,226]. Specifically, ER+ breast tumours in patients typically exhibit low or undetectable STAT1 expression levels and rarely possess activating KRAS mutations [225]. Moreover, mutations in the KRAS pathway can confer resistance to endocrine therapy in ER+ tumours, making these models less suitable for studying the effects of such treatments [228]. The *Wap-Cre;Pik3ca*$^{H1047R}$ model addresses this limitation, as PIK3CA mutations are commonly observed in human ER+ BC [22]. However, this model necessitates continuous oestradiol supplementation before and after tumour onset, which does not accurately represent the low physiological oestradiol levels found in post-menopausal patients [224].

The advent of whole genome sequencing technologies has significantly expanded our understanding of genetic tumour drivers, leading to the discovery of numerous new (breast) tumour drivers and suppressors. This progress contrasts sharply with the laborious process of generating GEMMs, which involves multiple steps of precise gene editing in Embryonic Stem Cells (ESCs), implantation into surrogate animals, germline transmission, genotyping, breeding, and colony expansion to produce an experimental cohort. Establishing concurrent genetic perturbations further extends this timeline by requiring repeated crossbreeding of strains with the desired genetic permutations. Ideally, a GEMM would develop tumours in specific tissues as a result of acquired somatic mutations, mirroring human carcinogenesis, with mutations arising post-development, puberty, and early adulthood. This requirement is only partially addressed by conditional models utilising Cre-recombinase or Tet-inducible allelic systems. Recently, Somatically Engineered Mouse Models (SEMMs) have emerged as a viable

alternative to traditional GEMMs. SEMMs enable genetic perturbations to be introduced into specific somatic cells of targeted organs *in vivo* [229]. This is typically achieved using viral vectors such as adeno-associated viruses (AAVs), adenoviruses (Ads), or lentiviral vectors (LVs) that express the gene of interest. By employing CRISPR/Cas9 technology, these vectors can be equipped with specific single guide RNAs (sgRNAs) to knockout any preferred gene in Cas9-tolerant mouse strains. The primary advantage of SEMMs over GEMMs is their significantly shorter generation time, as SEMMs require minimal breeding and genotyping, thus avoiding the disposal of mice with unfavourable genotypes. Furthermore, SEMMs enable tissue- and time-specific induction of tumourigenesis, closely mimicking human disease by targeting single somatic cells instead of the entire germline lineage. Lastly, SEMMs offer versatility in introducing genomic aberrations, facilitating higher throughput modelling approaches.

## Rat models

The concept of SEMMs is not formally limited to mouse models but can be extrapolated to other species (**Figure 4**) . Rats, in particular, have proven challenging to genetically engineer due to the difficulty of maintaining their embryonic stem cells (rESCs) *in vitro*, which has made pronuclear microinjections into rat oocytes inefficient. Subsequent embryo survival of successfully injected oocytes also presented itself with much lower incidence than previously observed in mice [230]. Improved ESC derivation and expansion protocols as well as the emergence of the CRISPR/Cas9 system allowed easier *in vitro* genetic manipulation of spermatogonial cells and subsequent establishment of germline mutations [231,232]. Due to its versatility, somatic engineering can readily be applied to rats as already demonstrated by Wang et al. and the subsequent rat 'infusion' models [233-235]. Compared to mice, rats are more similar to humans in terms of hormone receptor signalling and mammary gland architecture. Importantly, rats are known to develop breast carcinomas of ductal origin with lasting ER (and PR) expression, presenting, like the majority of human tumours, as luminal cancers responsive to oestrogen. These differences are hypothesised to result from species-specific pioneer factor usage. Mice exhibit species-specific differences in the distribution of FOXA1 motifs in ER binding sites, though the fundamental role of FOXA1 in hormone receptor signalling and mammary duct morphogenesis appear to be conserved between humans and mice [236]. Intriguingly, in mouse mammary glands, the transcription factor GATA3, an essential driver and prognostic biomarker in human luminal BC, is expressed at low levels [237,238]. In

contrast, rats express these factors and show greater similarity to human HR signalling, explaining their luminal tumour formation and treatment response [237-239]. Rats, in addition, have higher biological oestrogen levels than mice, where oestradiol is barely detectable [240,241]. Like humans, branched mammary ductal trees with delicate TDLU-like structures are seen in rats. These are believed to be the site of origin of human mammary carcinomas and appear to be absent in mice [201,242].

In the context of luminal BC research advances, the similarities between rat and human mammary gland biology, along with the ability to model pathologically similar luminal tumours, make rats a promising alternative for *in vivo* modelling, addressing several limitations of mouse models. All efforts undertaken to model luminal BC in rats were recently comprehensively described elsewhere [243].

## Conclusions and Discussion

Since the establishment of the first BC cell line in 1958, advances in experimental models, organoids, CDX/PDX biobanks, GEMMs, patient data acquisition, large-scale sequencing studies, and numerous clinical trials have profoundly expanded our understanding of BC. These innovations have allowed for characterisation of BC subtypes and illuminated the mechanisms underlying luminal BC biology, laying the foundation for personalised treatment approaches. Yet, despite these strides, critical challenges remain in improving patient outcomes.

The inherent heterogeneity of luminal BC poses significant challenges for clinical management, particularly in addressing therapy resistance and relapse. While molecular classifications have improved, our understanding of the biological drivers that differentiate luminal A from luminal B subtypes is still evolving. Emerging factors, such as the GR, have been implicated as key differentiators, but the exact mechanisms underlying these distinct behaviours remain elusive. Current transcriptomic classifications provide valuable prognostic information, but they fail to capture the functional complexity seen in patient disease trajectories. Large-scale genomic efforts have identified integrative clusters that hint at genomic alterations and intricate signalling interactions as critical factors in defining the aggressiveness and therapeutic response of luminal subtypes. However, the biological correlates

of these clusters remains poorly understood. To refine treatment regimens and accurately predict patient outcomes, we need a deeper understanding of these clusters and their impact on luminal A and B phenotypes.

The metastatic potential of luminal BC adds another layer of complexity to long-term disease management. Despite an initially favourable prognosis, luminal cancers are prone to late recurrences, often years or even decades after the initial diagnosis. This persistence is largely due to the ability of ER+ BC cells to enter a dormant state, evading therapy and leading to eventual relapse. Metabolic adaptations, epigenetic reprogramming, and cues from the tumour microenvironment may help sustain this dormancy. Understanding how the metastatic niche supports dormancy and facilitates reactivation is critical to advancing therapeutic strategies aimed at preventing relapse and managing metastasis effectively.

The tumour microenvironment plays a pivotal role in shaping nuclear receptor activity and luminal BC behaviour, including contributions from immune cells, fibroblasts, and metabolic factors. Ligands within distinct metastatic niches can either activate or suppress nuclear receptors, profoundly influencing tumour progression, dormancy, and therapy resistance. Understanding and targeting nuclear receptor cross-talk in various tumour microenvironments, including metastatic sites like bone, brain, and lung, could reveal novel therapeutic strategies. By disrupting nuclear receptor interactions or employing selective modulators, there is potential to overcome both dormancy and endocrine resistance, ultimately improving patient outcomes.

Moreover, endocrine resistance remains a formidable challenge in managing HR+ BC. The adaptability of ER signalling, influenced by co-regulators and other SHRs, contributes to both intrinsic and acquired resistance. Mechanisms such as reprogrammed ER binding, constitutively active ER mutants, and altered cross-talk with pathways like PI3K/AKT and MAPK drive resistance. Addressing these challenges requires identifying actionable targets within these pathways and developing effective combination therapies. Furthermore, broadening our understanding of the roles of nuclear receptors - including AR, GR, PPARγ, and LXRs - will be critical in addressing resistance and metastasis. Of interest, lifestyle factors like diet and exercise can modulate nuclear receptor activity potentially influencing their action and how they shape BC biology. Future research into the nutritional modulation of nuclear receptors, combined with targeted therapies, could broaden therapeutic strategies to

overcome endocrine resistance and enhance treatment outcomes for patients with luminal BC.

Experimental models are indispensable for advancing our understanding of these complex processes. While traditional cell line models have been foundational, they are limited in capturing the dynamic interplay between tumour progression and the microenvironment. Advanced models, including organoids, CDX/PDX models, GEMMs, and rat models with orthotopic tumour induction methods like MIND, show promise in more accurately replicating the biology of BC and preserving tumour characteristics. However, challenges persist in creating models that genuinely reflect the heterogeneity of primary luminal cancers, particularly in terms of molecular features, endocrine therapy response, and metastasis. Future efforts must focus on integrating diverse model systems and leveraging their unique strengths to bridge the gap between preclinical findings and clinical applications. Advances in genetic engineering, 3D cultures, and refined *in vivo* models will require collaborative efforts to standardise protocols, enhance reproducibility, and ensure that new insights effectively translate into clinical improvements. Integrating omics data - including genomics, epigenetics, transcriptomics, proteomics, and metabolomics - with advanced computational models holds the potential to unveil new therapeutic targets and address the heterogeneity of luminal BC. Furthermore, applying artificial intelligence and machine learning to these datasets can provide insights into patient-specific responses, helping to develop personalised treatment strategies. This integrated approach is essential for refining therapeutic interventions, mitigating recurrence, and ultimately improving survival outcomes for patients with HR+ BC.

## 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


1 Gudjonsson, T., Adriance, M. C., Sternlicht, M. D., Petersen, O. W. & Bissell, M. J. Myoepithelial Cells: Their Origin and Function in Breast Morphogenesis and Neoplasia. *J Mammary Gland Biol Neoplasia* **10**, 261-272, doi:10.1007/s10911-005-9586-4 (2005).

2 Shehata, M. *et al.* Phenotypic and functional characterisation of the luminal cell hierarchy of the mammary gland. *Breast Cancer Res* **14**, R134, doi:10.1186/bcr3334 (2012).

3 Foidart, J.-M. *et al.* Estradiol and Progesterone Regulate the Proliferation of Human Breast Epithelial Cells. *Fertility and Sterility* **69**, 963-969, doi:10.1016/S0015-0282(98)00042-9 (1998).

4 Brisken, C. & O'Malley, B. Hormone Action in the Mammary Gland. *Cold Spring Harbor Perspectives in Biology* **2**, a003178-a003178, doi:10.1101/cshperspect.a003178 (2010).

5 Bondesson, M., Hao, R., Lin, C.-Y., Williams, C. & Gustafsson, J.-Å. Estrogen receptor signaling during vertebrate development. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms* **1849**, 142-151, doi:10.1016/j.bbagrm.2014.06.005 (2015).

6 Hamilton, K. J., Hewitt, S. C., Arao, Y. & Korach, K. S. Estrogen Hormone Biology. *Curr Top Dev Biol* **125**, 109-146, doi:10.1016/bs.ctdb.2016.12.005 (2017).

7 Nilsson, S. *et al.* Mechanisms of Estrogen Action. *Physiological Reviews* **81**, 1535-1565, doi:10.1152/physrev.2001.81.4.1535 (2001).

8 Farage, M. A., Neill, S. & MacLean, A. B. Physiological Changes Associated with the Menstrual Cycle: A Review. *Obstetrical & Gynecological Survey* **64**, 58-72, doi:10.1097/OGX.0b013e3181932a37 (2009).

9 Ciwinska, M. *et al.* Mechanisms that clear mutations drive field cancerization in mammary tissue. *Nature* **633**, 198-206, doi:10.1038/s41586-024-07882-3 (2024).

10 Lim, E., Metzger-Filho, O. & Winer, E. P. The natural history of hormone receptor-positive breast cancer. *Oncology (Williston Park)* **26**, 688-694, 696 (2012).

11 Leone, J. P. *et al.* Factors associated with late risks of breast cancer-specific mortality in the SEER registry. *Breast Cancer Res Treat* **189**, 203-212, doi:10.1007/s10549-021-06233-4 (2021).

12 Hanker, A. B., Sudhan, D. R. & Arteaga, C. L. Overcoming Endocrine Resistance in Breast Cancer. *Cancer Cell* **37**, 496-513, doi:10.1016/j.ccell.2020.03.009 (2020).

13 Ciriello, G. *et al.* Comprehensive Molecular Portraits of Invasive Lobular Breast Cancer. *Cell* **163**, 506-519, doi:10.1016/j.cell.2015.09.033 (2015).

14 Dieci, M. V., Orvieto, E., Dominici, M., Conte, P. & Guarneri, V. Rare Breast Cancer Subtypes: Histological, Molecular, and Clinical Peculiarities. *The Oncologist* **19**, 805-813, doi:10.1634/theoncologist.2014-0108 (2014).

15 Ueng, S.-H., Mezzetti, T. & Tavassoli, F. A. Papillary Neoplasms of the Breast: A Review. *Archives of Pathology & Laboratory Medicine* **133**, 893-907, doi:10.5858/133.6.893 (2009).

16 Perou, C. M. *et al.* Molecular portraits of human breast tumours. *Nature* **406**, 747-752, doi:10.1038/35021093 (2000).

17 Sørlie, T. *et al.* Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 10869-10874, doi:10.1073/pnas.191367098 (2001).

18 Wallden, B. *et al.* Development and verification of the PAM50-based Prosigna breast cancer gene signature assay. *BMC Med Genomics* **8**, 54, doi:10.1186/s12920-015-0129-6 (2015).

19 Prat, A. *et al.* Prediction of Response to Neoadjuvant Chemotherapy Using Core Needle Biopsy Samples with the Prosigna Assay. *Clin Cancer Res* **22**, 560-566, doi:10.1158/1078-0432.Ccr-15-0630 (2016).

20 Ohnstad, H. O. *et al.* Impact of Prosigna test on adjuvant treatment decision in lymph node-negative early breast cancer-a prospective national multicentre study (EMIT-1). *ESMO Open* **9**, 103475, doi:10.1016/j.esmoop.2024.103475 (2024).

21 Group, M. *et al.* The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature* **486**, 346-352, doi:10.1038/nature10983 (2012).

22 The Cancer Genome Atlas Research, N. *et al.* The Cancer Genome Atlas Pan-Cancer analysis project. *Nat Genet* **45**, 1113-1120, doi:10.1038/ng.2764 (2013).

23 Braun, L. *et al.* Intrinsic breast cancer subtypes defined by estrogen receptor signalling—prognostic relevance of progesterone receptor loss. *Modern Pathology* **26**, 1161-1171, doi:10.1038/modpathol.2013.60 (2013).

24 Ades, F. *et al.* Luminal B Breast Cancer: Molecular Characterization, Clinical Management, and Future Perspectives. *JCO* **32**, 2794-2803, doi:10.1200/JCO.2013.54.1870 (2014).

25 Goldhirsch, A. *et al.* Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. *Ann Oncol* **24**, 2206-2223, doi:10.1093/annonc/mdt303 (2013).

26 Penault-Llorca, F. & Radosevic-Robin, N. Ki67 assessment in breast cancer: an update. *Pathology* **49**, 166-171, doi:10.1016/j.pathol.2016.11.006 (2017).

27 Polley, M.-Y. C. *et al.* An International Ki67 Reproducibility Study. *JNCI: Journal of the National Cancer Institute* **105**, 1897-1906, doi:10.1093/jnci/djt306 (2013).

28 Cardoso, F. *et al.* 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. *N Engl J Med* **375**, 717-729, doi:10.1056/NEJMoa1602253 (2016).

29 Filipits, M. *et al.* Prediction of Distant Recurrence Using EndoPredict Among Women with ER(+), HER2(-) Node-Positive and Node-Negative Breast Cancer Treated with Endocrine Therapy Only. *Clin Cancer Res* **25**, 3865-3872, doi:10.1158/1078-0432.Ccr-19-0376 (2019).

30 Sestak, I. *et al.* Prediction of chemotherapy benefit by EndoPredict in patients with breast cancer who received adjuvant endocrine therapy plus chemotherapy or endocrine therapy alone. *Breast Cancer Res Treat* **176**, 377-386, doi:10.1007/s10549-019-05226-8 (2019).

31 Paik, S. *et al.* A Multigene Assay to Predict Recurrence of Tamoxifen-Treated, Node-Negative Breast Cancer. *N Engl J Med* **351**, 2817-2826, doi:10.1056/NEJMoa041588 (2004).

32 Sparano, J. A. *et al.* Adjuvant Chemotherapy Guided by a 21-Gene Expression Assay in Breast Cancer. *N Engl J Med* **379**, 111-121, doi:10.1056/NEJMoa1804710 (2018).

33 Davis, B. A. *et al.* Racial and Ethnic Disparities in Oncotype DX Test Receipt in a Statewide Population-Based Study. *J Natl Compr Canc Netw* **15**, 346-354, doi:10.6004/jnccn.2017.0034 (2017).

34 Collin, L. J. *et al.* Oncotype DX recurrence score implications for disparities in chemotherapy and breast cancer mortality in Georgia. *npj Breast Cancer* **5**, 32, doi:10.1038/s41523-019-0129-3 (2019).

35 Prabhu, J. S. *et al.* A Majority of Low (1-10%) ER Positive Breast Cancers Behave Like Hormone Receptor Negative Tumors. *J Cancer* **5**, 156-165, doi:10.7150/jca.7668 (2014).

36 Schrodi, S. *et al.* Outcome of breast cancer patients with low hormone receptor positivity: analysis of a 15-year population-based cohort. *Annals of Oncology* **32**, 1410-1424, doi:10.1016/j.annonc.2021.08.1988 (2021).

37 Cejalvo, J. M. *et al.* Clinical implications of the non-luminal intrinsic subtypes in hormone receptor-positive breast cancer. *Cancer Treatment Reviews* **67**, 63-70, doi:10.1016/j.ctrv.2018.04.015 (2018).

38 Truong, T. H. & Lange, C. A. Deciphering Steroid Receptor Crosstalk in Hormone-Driven Cancers. *Endocrinology* **159**, 3897-3907, doi:10.1210/en.2018-00831 (2018).

39 Carson-Jurica, M. A., Schrader, W. T. & O'Malley, B. W. Steroid Receptor Family: Structure and Functions. *Endocrine Reviews* **11**, 201-220, doi:10.1210/edrv-11-2-201 (1990).

40 Weikum, E. R., Liu, X. & Ortlund, E. A. The nuclear receptor superfamily: A structural perspective. *Protein Science* **27**, 1876-1892, doi:10.1002/pro.3496 (2018).

41 Lavery, Derek N. & McEwan, Iain J. Structure and function of steroid receptor AF1 transactivation domains: induction of active conformations. *Biochemical Journal* **391**, 449-464, doi:10.1042/BJ20050872 (2005).

42 Wang, Q., Carroll, J. S. & Brown, M. Spatial and Temporal Recruitment of Androgen Receptor and Its Coactivators Involves Chromosomal Looping and Polymerase Tracking. *Molecular Cell* **19**, 631-642, doi:10.1016/j.molcel.2005.07.018 (2005).

43 D'Ippolito, A. M. *et al.* Pre-established Chromatin Interactions Mediate the Genomic Response to Glucocorticoids. *Cell Systems* **7**, 146-160.e147, doi:10.1016/j.cels.2018.06.007 (2018).

44 Prekovic, S. *et al.* Glucocorticoid receptor triggers a reversible drug-tolerant dormancy state with acquired therapeutic vulnerabilities in lung cancer. *Nat Commun* **12**, 4360, doi:10.1038/s41467-021-24537-3 (2021).

45 Berry, D. A. *et al.* Estrogen-Receptor Status and Outcomes of Modern Chemotherapy for Patients With Node-Positive Breast Cancer. *JAMA* **295**, 1658, doi:10.1001/jama.295.14.1658 (2006).

46 Patel, R., Klein, P., Tiersten, A. & Sparano, J. A. An emerging generation of endocrine therapies in breast cancer: a clinical perspective. *npj Breast Cancer* **9**, 20, doi:10.1038/s41523-023-00523-4 (2023).

47 Chi, D. *et al.* Estrogen receptor signaling is reprogrammed during breast tumorigenesis. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 11437-11443, doi:10.1073/pnas.1819155116 (2019).

48 Hurtado, A., Holmes, K. A., Ross-Innes, C. S., Schmidt, D. & Carroll, J. S. FOXA1 is a key determinant of estrogen receptor function and endocrine response. *Nat Genet* **43**, 27-33, doi:10.1038/ng.730 (2011).

49 Carroll, J. S. *et al.* Chromosome-Wide Mapping of Estrogen Receptor Binding Reveals Long-Range Regulation Requiring the Forkhead Protein FoxA1. *Cell* **122**, 33-43, doi:10.1016/j.cell.2005.05.008 (2005).

50 Theodorou, V., Stark, R., Menon, S. & Carroll, J. S. GATA3 acts upstream of FOXA1 in mediating ESR1 binding by shaping enhancer accessibility. *Genome Res.* **23**, 12-22, doi:10.1101/gr.139469.112 (2013).

51 Mohammed, H. *et al.* Progesterone receptor modulates ERα action in breast cancer. *Nature* **523**, 313-317, doi:10.1038/nature14583 (2015).

52 Scabia, V. *et al.* Estrogen receptor positive breast cancers have patient specific hormone sensitivities and rely on progesterone receptor. *Nat Commun* **13**, 3127, doi:10.1038/s41467-022-30898-0 (2022).

53 Truong, T. H. *et al.* Phosphorylated Progesterone Receptor Isoforms Mediate Opposing Stem Cell and Proliferative Breast Cancer Cell Fates. *Endocrinology* **160**, 430-446, doi:10.1210/en.2018-00990 (2019).

54 Mayayo-Peralta, I., Prekovic, S. & Zwart, W. Estrogen Receptor on the move: Cistromic plasticity and its implications in breast cancer. *Molecular Aspects of Medicine* **78**, 100939, doi:10.1016/j.mam.2020.100939 (2021).

55 Severson, T. M. *et al.* Characterizing steroid hormone receptor chromatin binding landscapes in male and female breast cancer. *Nat Commun* **9**, 482, doi:10.1038/s41467-018-02856-2 (2018).

56 Severson, T. M. *et al.* Neoadjuvant tamoxifen synchronizes ERα binding and gene expression profiles related to outcome and proliferation. *Oncotarget* **7**, 33901-33918, doi:10.18632/oncotarget.8983 (2016).

57 Ross-Innes, C. S. *et al.* Differential oestrogen receptor binding is associated with clinical outcome in breast cancer. *Nature* **481**, 389-393, doi:10.1038/nature10730 (2012).

58 Martin, L.-A. *et al.* Discovery of naturally occurring ESR1 mutations in breast cancer cell lines modelling endocrine resistance. *Nat Commun* **8**, 1865, doi:10.1038/s41467-017-01864-y (2017).

59 Vitale, S. R. *et al.* The prognostic and predictive value of ESR1 fusion gene transcripts in primary breast cancer. *BMC Cancer* **22**, 165, doi:10.1186/s12885-022-09265-1 (2022).

60 Li, L. *et al.* Therapeutic role of recurrent ESR1-CCDC170 gene fusions in breast cancer endocrine resistance. *Breast Cancer Res* **22**, 84, doi:10.1186/s13058-020-01325-3 (2020).

61 Harrod, A. *et al.* Genome engineering for estrogen receptor mutations reveals differential responses to anti-estrogens and new prognostic gene signatures for breast cancer. *Oncogene* **41**, 4905-4915, doi:10.1038/s41388-022-02483-8 (2022).

62 Alves, C. L. *et al.* Co-targeting CDK4/6 and AKT with endocrine therapy prevents progression in CDK4/6 inhibitor and endocrine therapy-resistant breast cancer. *Nat Commun* **12**, 5112, doi:10.1038/s41467-021-25422-9 (2021).

63 Rugo, H. S., Vidula, N. & Ma, C. Improving Response to Hormone Therapy in Breast Cancer: New Targets, New Therapeutic Options. *American Society of Clinical Oncology Educational Book*, e40-e54, doi:10.1200/EDBK_159198 (2016).

64 Wintermantel, T. M., Bock, D., Fleig, V., Greiner, E. F. & Schütz, G. The Epithelial Glucocorticoid Receptor Is Required for the Normal Timing of Cell Proliferation during Mammary Lobuloalveolar Development but Is Dispensable for Milk Production. *Molecular Endocrinology* **19**, 340-349, doi:10.1210/me.2004-0068 (2005).

65 Tronche, F., Kellendonk, C., Reichardt, H. M. & Schütz, G. Genetic dissection of glucocorticoid receptor function in mice. *Current Opinion in Genetics & Development* **8**, 532-538, doi:10.1016/S0959-437X(98)80007-5 (1998).

66 Lien, H. C. *et al.* Differential expression of glucocorticoid receptor in human breast tissues and related neoplasms. *The Journal of Pathology* **209**, 317-327, doi:10.1002/path.1982 (2006).

67 Buxant, F., Engohan-Aloghe, C. & Noël, J.-C. Estrogen Receptor, Progesterone Receptor, and Glucocorticoid Receptor Expression in Normal Breast Tissue, Breast In Situ Carcinoma, and Invasive Breast Cancer. *Applied Immunohistochemistry & Molecular Morphology* **18**, 254-257, doi:10.1097/PAI.0b013e3181c10180 (2010).

68 Osborne, C. K. *et al.* Direct inhibition of growth and antagonism of insulin action by glucocorticoids in human breast cancer cells in culture. *Cancer Res* **39**, 2422-2428 (1979).

69 Wu, W. *et al.* Microarray Analysis Reveals Glucocorticoid-Regulated Survival Genes That Are Associated With Inhibition of Apoptosis in Breast Epithelial Cells. *Cancer Research* **64**, 1757-1764, doi:10.1158/0008-5472.CAN-03-2546 (2004).

70 Prekovic, S. *et al.* Luminal breast cancer identity is determined by loss of glucocorticoid receptor activity. *EMBO Mol Med* **15**, e17737, doi:10.15252/emmm.202317737 (2023).

71 Mayayo-Peralta, I., Zwart, W. & Prekovic, S. Duality of glucocorticoid action in cancer: tumor-suppressor or oncogene? *Endocrine-Related Cancer* **28**, R157-R171, doi:10.1530/ERC-20-0489 (2021).

72 West, D. C. *et al.* GR and ER Coactivation Alters the Expression of Differentiation Genes and Associates with Improved ER+ Breast Cancer Outcome. *Molecular Cancer Research* **14**, 707-719, doi:10.1158/1541-7786.MCR-15-0433 (2016).

73 Karmakar, S., Jin, Y. & Nagaich, A. K. Interaction of Glucocorticoid Receptor (GR) with Estrogen Receptor (ER) α and Activator Protein 1 (AP1) in Dexamethasone-mediated Interference of ERα Activity. *Journal of Biological Chemistry* **288**, 24020-24034, doi:10.1074/jbc.M113.473819 (2013).

74 Zhang, C. *et al.* Corticosteroids induce chemotherapy resistance in the majority of tumour cells from bone, brain, breast, cervix, melanoma and neuroblastoma. *Int J Oncol*, doi:10.3892/ijo.29.5.1295 (2006).

75 Colombo, N. *et al.* Relacorilant + Nab-Paclitaxel in Patients With Recurrent, Platinum-Resistant Ovarian Cancer: A Three-Arm, Randomized, Controlled, Open-Label Phase II Study. *JCO* **41**, 4779-4789, doi:10.1200/JCO.22.02624 (2023).

76 Prekovic, S. & Zwart, W. Inhibiting the Glucocorticoid Receptor to Enhance Chemotherapy Response. *JCO* **41**, 4790-4793, doi:10.1200/JCO.23.01195 (2023).

77 Dimitrakakis, C., Zhou, J. & Bondy, C. A. Androgens and mammary growth and neoplasia. *Fertility and Sterility* **77**, 26-33, doi:10.1016/S0015-0282(02)02979-5 (2002).

78 Raths, F. *et al.* The molecular consequences of androgen activity in the human breast. *Cell Genomics* **3**, 100272, doi:10.1016/j.xgen.2023.100272 (2023).

79 Yu, Q. *et al.* Expression of androgen receptor in breast cancer and its significance as a prognostic factor. *Annals of Oncology* **22**, 1288-1294, doi:10.1093/annonc/mdq586 (2011).

80 Kensler, K. H. *et al.* Androgen Receptor Expression and Breast Cancer Survival: Results From the Nurses' Health Studies. *J Natl Cancer Inst* **111**, 700-708, doi:10.1093/jnci/djy173 (2019).

81 Hickey, T. E. *et al.* The androgen receptor is a tumor suppressor in estrogen receptor–positive breast cancer. *Nat Med* **27**, 310-320, doi:10.1038/s41591-020-01168-7 (2021).

82 Hosseinzadeh, L. *et al.* The androgen receptor interacts with GATA3 to transcriptionally regulate a luminal epithelial cell phenotype in breast cancer. *Genome Biol* **25**, 44, doi:10.1186/s13059-023-03161-y (2024).

83 Coss, C. C., Jones, A. & Dalton, J. T. Selective androgen receptor modulators as improved androgen therapy for advanced breast cancer. *Steroids* **90**, 94-100, doi:10.1016/j.steroids.2014.06.010 (2014).

84 Jordan, V. C. *Estrogen Action, Selective Estrogen Receptor Modulators and Women's Health*.

85 Palmieri, C. *et al.* Activity and safety of enobosarm, a novel, oral, selective androgen receptor modulator, in androgen receptor-positive, oestrogen receptor-positive, and HER2-negative advanced breast cancer (Study G200802): a randomised, open-label, multicentre, multinational, parallel design, phase 2 trial. *The Lancet Oncology* **25**, 317-325, doi:10.1016/S1470-2045(24)00004-4 (2024).

86 Cochrane, D. R. *et al.* Role of the androgen receptor in breast cancer and preclinical analysis of enzalutamide. *Breast Cancer Res* **16**, R7, doi:10.1186/bcr3599 (2014).

87 Elias, A. D. *et al.* Phase II trial of fulvestrant plus enzalutamide in ER+/HER2– advanced breast cancer. *npj Breast Cancer* **9**, 41, doi:10.1038/s41523-023-00544-z (2023).

88 Wei, L. *et al.* Pharmacological Targeting of Androgen Receptor Elicits Context-Specific Effects in Estrogen Receptor–Positive Breast Cancer. *Cancer Research* **83**, 456-470, doi:10.1158/0008-5472.CAN-22-1016 (2023).

89 Vedin, L.-L., Lewandowski, S. A., Parini, P., Gustafsson, J.-Å. & Steffensen, K. R. The oxysterol receptor LXR inhibits proliferation of human breast cancer cells. *Carcinogenesis* **30**, 575-579, doi:10.1093/carcin/bgp029 (2009).

90 Li, D.-H. *et al.* PPARG: A Promising Therapeutic Target in Breast Cancer and Regulation by Natural Drugs. *PPAR Research* **2023**, 1-18, doi:10.1155/2023/4481354 (2023).

91 Swales, K. E. *et al.* The Farnesoid X Receptor Is Expressed in Breast Cancer and Regulates Apoptosis and Aromatase Expression. *Cancer Research* **66**, 10120-10126, doi:10.1158/0008-5472.CAN-06-2399 (2006).

92 Giordano, C. *et al.* Farnesoid X receptor inhibits tamoxifen-resistant MCF-7 breast cancer cell growth through downregulation of HER2 expression. *Oncogene* **30**, 4129-4140, doi:10.1038/onc.2011.124 (2011).

93 Loo, S. Y. *et al.* Epigenetic derepression converts PPARγ into a druggable target in triple-negative and endocrine-resistant breast cancers. *Cell Death Discov.* **7**, 265, doi:10.1038/s41420-021-00635-5 (2021).

94 Du, T. *et al.* Key regulators of lipid metabolism drive endocrine resistance in invasive lobular breast cancer. *Breast Cancer Res* **20**, 106, doi:10.1186/s13058-018-1041-8 (2018).

95 Laganière, J. e., Deblois, G. v. & Giguère, V. Functional Genomics Identifies a Mechanism for Estrogen Activation of the Retinoic Acid Receptor α1 Gene in Breast Cancer Cells. *Molecular Endocrinology* **19**, 1584-1592, doi:10.1210/me.2005-0040 (2005).

96 Hua, S., Kittler, R. & White, K. P. Genomic Antagonism between Retinoic Acid and Estrogen Signaling in Breast Cancer. *Cell* **137**, 1259-1271, doi:10.1016/j.cell.2009.04.043 (2009).

97 Vanhevel, J., Verlinden, L., Doms, S., Wildiers, H. & Verstuyf, A. The role of vitamin D in breast cancer risk and progression. *Endocrine-Related Cancer* **29**, R33-R55, doi:10.1530/ERC-21-0182 (2022).

98 Manna, S. *et al.* ERRα Is a Marker of Tamoxifen Response and Survival in Triple-Negative Breast Cancer. *Clinical Cancer Research* **22**, 1421-1431, doi:10.1158/1078-0432.CCR-15-0857 (2016).

99 Ijichi, N. *et al.* Estrogen-related receptor γ modulates cell proliferation and estrogen signaling in breast cancer. *The Journal of Steroid Biochemistry and Molecular Biology* **123**, 1-7, doi:10.1016/j.jsbmb.2010.09.002 (2011).

100 Stein, R. A. *et al.* Estrogen-Related Receptor α Is Critical for the Growth of Estrogen Receptor–Negative Breast Cancer. *Cancer Research* **68**, 8805-8812, doi:10.1158/0008-5472.CAN-08-1594 (2008).

101 Thewes, V. *et al.* Reprogramming of the ERRα and ERα Target Gene Landscape Triggers Tamoxifen Resistance in Breast Cancer. *Cancer Research* **75**, 720-731, doi:10.1158/0008-5472.CAN-14-0652 (2015).

102 Park, S. *et al.* ERRα-Regulated Lactate Metabolism Contributes to Resistance to Targeted Therapies in Breast Cancer. *Cell Reports* **15**, 323-335, doi:10.1016/j.celrep.2016.03.026 (2016).

103 Rueda, O. M. *et al.* Dynamics of breast-cancer relapse reveal late-recurring ER-positive genomic subgroups. *Nature* **567**, 399-404, doi:10.1038/s41586-019-1007-8 (2019).

104 Pan, H. *et al.* 20-Year Risks of Breast-Cancer Recurrence after Stopping Endocrine Therapy at 5 Years. *N Engl J Med* **377**, 1836-1846, doi:10.1056/NEJMoa1701830 (2017).

105 Hanahan, D. Hallmarks of Cancer: New Dimensions. *Cancer Discovery* **12**, 31-46, doi:10.1158/2159-8290.CD-21-1059 (2022).

106 Hebert, J. D., Neal, J. W. & Winslow, M. M. Dissecting metastasis using preclinical models and methods. *Nat Rev Cancer* **23**, 391-407, doi:10.1038/s41568-023-00568-4 (2023).

107 Massagué, J. & Ganesh, K. Metastasis-Initiating Cells and Ecosystems. *Cancer Discovery* **11**, 971-994, doi:10.1158/2159-8290.CD-21-0010 (2021).

108 Gkountela, S. *et al.* Circulating Tumor Cell Clustering Shapes DNA Methylation to Enable Metastasis Seeding. *Cell* **176**, 98-112.e114, doi:10.1016/j.cell.2018.11.046 (2019).

109 Padmanaban, V. *et al.* E-cadherin is required for metastasis in multiple models of breast cancer. *Nature* **573**, 439-444, doi:10.1038/s41586-019-1526-3 (2019).

110 Szczerba, B. M. *et al.* Neutrophils escort circulating tumour cells to enable cell cycle progression. *Nature* **566**, 553-557, doi:10.1038/s41586-019-0915-y (2019).

111 Luzzi, K. J. *et al.* Multistep Nature of Metastatic Inefficiency. *The American Journal of Pathology* **153**, 865-873, doi:10.1016/S0002-9440(10)65628-3 (1998).

112 Kienast, Y. *et al.* Real-time imaging reveals the single steps of brain metastasis formation. *Nat Med* **16**, 116-122, doi:10.1038/nm.2072 (2010).

113 Wikman, H., Vessella, R. & Pantel, K. Cancer micrometastasis and tumour dormancy. *APMIS* **116**, 754-770, doi:10.1111/j.1600-0463.2008.01033.x (2008).

114 Rosano, D. *et al.* Long-term Multimodal Recording Reveals Epigenetic Adaptation Routes in Dormant Breast Cancer Cells. *Cancer Discovery* **14**, 866-889, doi:10.1158/2159-8290.CD-23-1161 (2024).

115 Patten, D. K. *et al.* Enhancer mapping uncovers phenotypic heterogeneity and evolution in patients with luminal breast cancer. *Nat Med* **24**, 1469-1480, doi:10.1038/s41591-018-0091-x (2018).

116 Johnson, Rachelle W. *et al.* Induction of LIFR confers a dormancy phenotype in breast cancer cells disseminated to the bone marrow. *Nat Cell Biol* **18**, 1078-1089, doi:10.1038/ncb3408 (2016).

117 Hampsch, R. A. *et al.* AMPK Activation by Metformin Promotes Survival of Dormant ER+ Breast Cancer Cells. *Clinical Cancer Research* **26**, 3707-3719, doi:10.1158/1078-0432.CCR-20-0269 (2020).

118 Kronblad, Å., Hedenfalk, I., Nilsson, E., Påhlman, S. & Landberg, G. ERK1/2 inhibition increases antiestrogen treatment efficacy by interfering with hypoxia-induced downregulation of ERα: a combination therapy potentially targeting hypoxic and dormant tumor cells. *Oncogene* **24**, 6835-6841, doi:10.1038/sj.onc.1208830 (2005).

119 Carlson, P. *et al.* Targeting the perivascular niche sensitizes disseminated tumour cells to chemotherapy. *Nat Cell Biol* **21**, 238-250, doi:10.1038/s41556-018-0267-0 (2019).

120 Barkan, D. *et al.* Metastatic Growth from Dormant Cells Induced by a Col-I–Enriched Fibrotic Environment. *Cancer Research* **70**, 5706-5716, doi:10.1158/0008-5472.CAN-09-2356 (2010).

121 Han, H. H. *et al.* Angiopoietin-2 promotes ER+ breast cancer cell survival in bone marrow niche. *Endocrine-Related Cancer* **23**, 609-623, doi:10.1530/ERC-16-0086 (2016).

122 Moreira, P. I., Custódio, J., Moreno, A., Oliveira, C. R. & Santos, M. S. Tamoxifen and Estradiol Interact with the Flavin Mononucleotide Site of Complex I Leading to Mitochondrial Failure. *Journal of Biological Chemistry* **281**, 10143-10152, doi:10.1074/jbc.M510249200 (2006).

123 Sansone, P. *et al.* Packaging and transfer of mitochondrial DNA via exosomes regulate escape from dormancy in hormonal therapy-resistant breast cancer. *Proc. Natl. Acad. Sci. U.S.A.* **114**, doi:10.1073/pnas.1704862114 (2017).

124 Gawrzak, S. *et al.* Publisher Correction: MSK1 regulates luminal cell differentiation and metastatic dormancy in ER+ breast cancer. *Nat Cell Biol* **20**, 990-990, doi:10.1038/s41556-018-0052-0 (2018).

125 Aguirre-Ghiso, J. A., Estrada, Y., Liu, D. & Ossowski, L. ERK(MAPK) activity as a determinant of tumor growth and dormancy; regulation by p38(SAPK). *Urologic Oncology: Seminars and Original Investigations* **22**, 82, doi:10.1016/j.urolonc.2003.12.012 (2004).

126 Aguirre-Ghiso, J. A., Ossowski, L. & Rosenbaum, S. K. Green Fluorescent Protein Tagging of Extracellular Signal-Regulated Kinase and p38 Pathways Reveals Novel Dynamics of Pathway Activation during Primary and Metastatic Growth. *Cancer Research* **64**, 7336-7345, doi:10.1158/0008-5472.CAN-04-0113 (2004).

127 Llorente, A. *et al.* MAF amplification licenses ERα through epigenetic remodelling to drive breast cancer metastasis. *Nat Cell Biol* **25**, 1833-1847, doi:10.1038/s41556-023-01281-y (2023).

128 Cejalvo, J. M. *et al.* Intrinsic Subtypes and Gene Expression Profiles in Primary and Metastatic Breast Cancer. *Cancer Research* **77**, 2213-2221, doi:10.1158/0008-5472.CAN-16-2717 (2017).

129 Garcia-Recio, S. *et al.* Multiomics in primary and metastatic breast tumors from the AURORA US network finds microenvironment and epigenetic drivers of metastasis. *Nat Cancer*, doi:10.1038/s43018-022-00491-x (2022).

130 Garcia-Recio, S. *et al.* FGFR4 regulates tumor subtype differentiation in luminal breast cancer and metastatic disease. *Journal of Clinical Investigation* **130**, 4871-4887, doi:10.1172/JCI130323 (2020).

131 Cosgrove, N. *et al.* Mapping molecular subtype specific alterations in breast cancer brain metastases identifies clinically relevant vulnerabilities. *Nat Commun* **13**, 514, doi:10.1038/s41467-022-27987-5 (2022).

132 Yates, L. R. *et al.* Genomic Evolution of Breast Cancer Metastasis and Relapse. *Cancer Cell* **32**, 169-184.e167, doi:10.1016/j.ccell.2017.07.005 (2017).

133 Varešlija, D. *et al.* Transcriptome Characterization of Matched Primary Breast and Brain Metastatic Tumors to Detect Novel Actionable Targets. *JNCI: Journal of the National Cancer Institute* **111**, 388-398, doi:10.1093/jnci/djy110 (2019).

134 Mayayo-Peralta, I. *et al.* Proteomics on malignant pleural effusions reveals ERα loss in metastatic breast cancer associates with SGK1-NDRG1 deregulation. *Mol Oncol* **18**, 156-169, doi:10.1002/1878-0261.13540 (2024).

135 Nagarajan, S. *et al.* ARID1A influences HDAC1/BRD4 activity, intrinsic proliferative capacity and breast cancer treatment response. *Nat Genet* **52**, 187-197, doi:10.1038/s41588-019-0541-5 (2020).

136 Nguyen, B. *et al.* Genomic characterization of metastatic patterns from prospective clinical sequencing of 25,000 patients. *Cell* **185**, 563-575.e511, doi:10.1016/j.cell.2022.01.003 (2022).

137 Angus, L. *et al.* The genomic landscape of metastatic breast cancer highlights changes in mutation and signature frequencies. *Nat Genet* **51**, 1450-1458, doi:10.1038/s41588-019-0507-7 (2019).

138 Mosele, F. *et al.* Outcome and molecular landscape of patients with PIK3CA-mutated metastatic breast cancer. *Annals of Oncology* **31**, 377-386, doi:10.1016/j.annonc.2019.11.006 (2020).

139 Paul, M. R. *et al.* Genomic landscape of metastatic breast cancer identifies preferentially dysregulated pathways and targets. *Journal of Clinical Investigation*, 10.1172/JCI129941, doi:10.1172/JCI129941 (2020).

140 Toy, W. *et al.* ESR1 ligand-binding domain mutations in hormone-resistant breast cancer. *Nat Genet* **45**, 1439-1445, doi:10.1038/ng.2822 (2013).

141 Jeselsohn, R. *et al.* Emergence of Constitutively Active Estrogen Receptor-α Mutations in Pretreated Advanced Estrogen Receptor–Positive Breast Cancer. *Clinical Cancer Research* **20**, 1757-1767, doi:10.1158/1078-0432.CCR-13-2332 (2014).

142 Chandarlapaty, S. *et al.* Prevalence of ESR1 Mutations in Cell-Free DNA and Outcomes in Metastatic Breast Cancer: A Secondary Analysis of the BOLERO-2 Clinical Trial. *JAMA Oncol* **2**, 1310, doi:10.1001/jamaoncol.2016.1279 (2016).

143 Will, M., Liang, J., Metcalfe, C. & Chandarlapaty, S. Therapeutic resistance to anti-oestrogen therapy in breast cancer. *Nat Rev Cancer* **23**, 673-685, doi:10.1038/s41568-023-00604-3 (2023).

144 Razavi, P. *et al.* The Genomic Landscape of Endocrine-Resistant Advanced Breast Cancers. *Cancer Cell* **34**, 427-438.e426, doi:10.1016/j.ccell.2018.08.008 (2018).

145 Li, S. *et al.* Endocrine-Therapy-Resistant ESR1 Variants Revealed by Genomic Characterization of Breast-Cancer-Derived Xenografts. *Cell Reports* **4**, 1116-1130, doi:10.1016/j.celrep.2013.08.022 (2013).

146 Zhao, Y. *et al.* Structurally Novel Antiestrogens Elicit Differential Responses from Constitutively Active Mutant Estrogen Receptors in Breast Cancer Cells and Tumors. *Cancer Research* **77**, 5602-5613, doi:10.1158/0008-5472.CAN-17-1265 (2017).

147 Magnani, L. *et al.* Acquired CYP19A1 amplification is an early specific mechanism of aromatase inhibitor resistance in ERα metastatic breast cancer. *Nat Genet* **49**, 444-450, doi:10.1038/ng.3773 (2017).

148 Sokol, E. S. *et al.* Loss of function of NF1 is a mechanism of acquired resistance to endocrine therapy in lobular breast cancer. *Ann Oncol* **30**, 115-123, doi:10.1093/annonc/mdy497 (2019).

149 Razavi, P. *et al.* Alterations in PTEN and ESR1 promote clinical resistance to alpelisib plus aromatase inhibitors. *Nat Cancer* **1**, 382-393, doi:10.1038/s43018-020-0047-1 (2020).

150 Ran, R. *et al.* A role for CBFβ in maintaining the metastatic phenotype of breast cancer cells. *Oncogene* **39**, 2624-2637, doi:10.1038/s41388-020-1170-2 (2020).

151 Bado, I., Gugala, Z., Fuqua, S. A. W. & Zhang, X. H. F. Estrogen receptors in breast and bone: from virtue of remodeling to vileness of metastasis. *Oncogene* **36**, 4527-4537, doi:10.1038/onc.2017.94 (2017).

152 Fehm, T. *et al.* ERalpha-status of disseminated tumour cells in bone marrow of primary breast cancer patients. *Breast Cancer Res* **10**, R76, doi:10.1186/bcr2143 (2008).

153 Hoefnagel, L. D. C. *et al.* Discordance in ERα, PR and HER2 receptor status across different distant breast cancer metastases within the same patient. *Annals of Oncology* **24**, 3017-3023, doi:10.1093/annonc/mdt390 (2013).

154 Eyre, R. *et al.* Microenvironmental IL1β promotes breast cancer metastatic colonisation in the bone via activation of Wnt signalling. *Nat Commun* **10**, 5016, doi:10.1038/s41467-019-12807-0 (2019).

155 Nobre, A. R. *et al.* Bone marrow NG2+/Nestin+ mesenchymal stem cells drive DTC dormancy via TGF-β2. *Nat Cancer* **2**, 327-339, doi:10.1038/s43018-021-00179-8 (2021).

156 Wang, H. *et al.* The Osteogenic Niche Is a Calcium Reservoir of Bone Micrometastases and Confers Unexpected Therapeutic Vulnerability. *Cancer Cell* **34**, 823-839.e827, doi:10.1016/j.ccell.2018.10.002 (2018).

157 Braun, S. *et al.* A Pooled Analysis of Bone Marrow Micrometastasis in Breast Cancer. *N Engl J Med* **353**, 793-802, doi:10.1056/NEJMoa050434 (2005).

158 Magbanua, M. J. M. *et al.* Outcomes and clinicopathologic characteristics associated with disseminated tumor cells in bone marrow after neoadjuvant chemotherapy in high-risk early stage breast cancer: the I-SPY SURMOUNT study. *Breast Cancer Res Treat* **198**, 383-390, doi:10.1007/s10549-022-06803-0 (2023).

159 Bado, I. L. *et al.* The bone microenvironment increases phenotypic plasticity of ER+ breast cancer cells. *Developmental Cell* **56**, 1100-1117.e1109, doi:10.1016/j.devcel.2021.03.008 (2021).

160 Zhang, W. *et al.* The bone microenvironment invigorates metastatic seeds for further dissemination. *Cell* **184**, 2471-2486.e2420, doi:10.1016/j.cell.2021.03.011 (2021).

161 Keelan, S. *et al.* Dynamic epi-transcriptomic landscape mapping with disease progression in estrogen receptor-positive breast cancer. *Cancer Communications* **43**, 615-619, doi:10.1002/cac2.12407 (2023).

162 Pecar, G. *et al.* RET signaling in breast cancer therapeutic resistance and metastasis. *Breast Cancer Res* **25**, 26, doi:10.1186/s13058-023-01622-7 (2023).

163 Jagust, P. *et al.* RET overexpression leads to increased brain metastatic competency in luminal breast cancer. *JNCI: Journal of the National Cancer Institute* **116**, 1632-1644, doi:10.1093/jnci/djae091 (2024).

164 Ward, E. *et al.* Epigenome-wide SRC-1–Mediated Gene Silencing Represses Cellular Differentiation in Advanced Breast Cancer. *Clinical Cancer Research* **24**, 3692-3703, doi:10.1158/1078-0432.CCR-17-2615 (2018).

165 Charmsaz, S. *et al.* ADAM22/LGI1 complex as a new actionable target for breast cancer brain metastasis. *BMC Med* **18**, 349, doi:10.1186/s12916-020-01806-4 (2020).

166 Varešlija, D. *et al.* Comparative analysis of the AIB1 interactome in breast cancer reveals MTA2 as a repressive partner which silences E-Cadherin to promote EMT and associates with a pro-metastatic phenotype. *Oncogene* **40**, 1318-1331, doi:10.1038/s41388-020-01606-3 (2021).

167 Albrengues, J. *et al.* Neutrophil extracellular traps produced during inflammation awaken dormant cancer cells in mice. *Science* **361**, eaao4227, doi:10.1126/science.aao4227 (2018).

168 Pedersen, R. N., Mellemkjær, L., Ejlertsen, B., Nørgaard, M. & Cronin-Fenton, D. Abstract P3-12-18: Mortality after late breast cancer recurrence in Denmark. *Cancer Research* **82**, P3-12-18-P13-12-18, doi:10.1158/1538-7445.SABCS21-P3-12-18 (2022).

169 Liu, Z. *et al.* Young age at diagnosis is associated with worse prognosis in the Luminal A breast cancer subtype: a retrospective institutional cohort study. *Breast Cancer Res Treat* **172**, 689-702, doi:10.1007/s10549-018-4950-4 (2018).

170 Turrell, F. K. *et al.* Age-associated microenvironmental changes highlight the role of PDGF-C in ER+ breast cancer metastatic relapse. *Nat Cancer* **4**, 468-484, doi:10.1038/s43018-023-00525-y (2023).

171 Gazdar, A. F. *et al.* Characterization of paired tumor and non-tumor cell lines established from patients with breast cancer. *International Journal of Cancer* **78**, 766-774, doi:10.1002/(SICI)1097-0215(19981209)78:6<766::AID-IJC15>3.0.CO;2-L (1998).

172 Cailleau, R., Olivé, M. & Cruciger, Q. V. J. Long-term human breast carcinoma cell lines of metastatic origin: Preliminary characterization. *In Vitro* **14**, 911-915, doi:10.1007/BF02616120 (1978).

173 Lasfargues, E. Y. & Ozzello, L. Cultivation of human breast carcinomas. *J Natl Cancer Inst* **21**, 1131-1147 (1958).

174 Sharma, M. P., Shukla, S. & Misra, G. Recent advances in breast cancer cell line research. *International Journal of Cancer* **154**, 1683-1693, doi:10.1002/ijc.34849 (2024).

175 Witt, B. L. & Tollefsbol, T. O. Molecular, Cellular, and Technical Aspects of Breast Cancer Cell Lines as a Foundational Tool in Cancer Research. *Life* **13**, 2311, doi:10.3390/life13122311 (2023).

176 Barretina, J. *et al.* The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. *Nature* **483**, 603-607, doi:10.1038/nature11003 (2012).

177 Garnett, M. J. *et al.* Systematic identification of genomic markers of drug sensitivity in cancer cells. *Nature* **483**, 570-575, doi:10.1038/nature11005 (2012).

178 Ethier, S. P. Growth Factor Synthesis and Human Breast Cancer Progression. *JNCI Journal of the National Cancer Institute* **87**, 964-973, doi:10.1093/jnci/87.13.964 (1995).

179 Dai, X., Cheng, H., Bai, Z. & Li, J. Breast Cancer Cell Line Classification and Its Relevance with Breast Tumor Subtyping. *J. Cancer* **8**, 3131-3141, doi:10.7150/jca.18457 (2017).

180 Neve, R. M. *et al.* A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. *Cancer Cell* **10**, 515-527, doi:10.1016/j.ccr.2006.10.008 (2006).

181 Forozan, F. *et al.* Comparative genomic hybridization analysis of 38 breast cancer cell lines: a basis for interpreting complementary DNA microarray data. *Cancer Res* **60**, 4519-4525 (2000).

182 Hiscox, S. *et al.* Overexpression of CD44 accompanies acquired tamoxifen resistance in MCF7 cells and augments their sensitivity to the stromal factors, heregulin and hyaluronan. *BMC Cancer* **12**, 458, doi:10.1186/1471-2407-12-458 (2012).

183 Riaz, M. *et al.* miRNA expression profiling of 51 human breast cancer cell lines reveals subtype and driver mutation-specific miRNAs. *Breast Cancer Res* **15**, R33, doi:10.1186/bcr3415 (2013).

184 Hollestelle, A. *et al.* Distinct gene mutation profiles among luminal-type and basal-type breast cancer cell lines. *Breast Cancer Res Treat* **121**, 53-64, doi:10.1007/s10549-009-0460-8 (2010).

185 Kao, J. *et al.* Molecular profiling of breast cancer cell lines defines relevant tumor models and provides a resource for cancer gene discovery. *PLoS One* **4**, e6146, doi:10.1371/journal.pone.0006146 (2009).

186 Dvořánková, B. *et al.* Fibroblasts prepared from different types of malignant tumors stimulate expression of luminal marker keratin 8 in the EM-G3 breast cancer cell line. *Histochem Cell Biol* **137**, 679-685, doi:10.1007/s00418-012-0918-3 (2012).

187 Briand, P. & Lykkesfeldt, A. E. An in vitro Model of Human Breast Carcinogenesis: Epigenetic Aspects. *Breast Cancer Res Treat* **65**, 179-187, doi:10.1023/A:1006434503061 (2001).

188 Kim, J., Koo, B.-K. & Knoblich, J. A. Human organoids: model systems for human biology and medicine. *Nat Rev Mol Cell Biol* **21**, 571-584, doi:10.1038/s41580-020-0259-3 (2020).

189 Linnemann, J. R. *et al.* Quantification of regenerative potential in primary human mammary epithelial cells. *Development*, dev.123554, doi:10.1242/dev.123554 (2015).

190 Sachs, N. *et al.* A Living Biobank of Breast Cancer Organoids Captures Disease Heterogeneity. *Cell* **172**, 373-386.e310, doi:10.1016/j.cell.2017.11.010 (2018).

191 Dekkers, J. F. *et al.* Long-term culture, genetic manipulation and xenotransplantation of human normal and breast cancer organoids. *Nat Protoc* **16**, 1936-1965, doi:10.1038/s41596-020-00474-1 (2021).

192 Guillen, K. P. *et al.* A human breast cancer-derived xenograft and organoid platform for drug discovery and precision oncology. *Nat Cancer* **3**, 232-250, doi:10.1038/s43018-022-00337-6 (2022).

193 Souto, E. P., Dobrolecki, L. E., Villanueva, H., Sikora, A. G. & Lewis, M. T. In Vivo Modeling of Human Breast Cancer Using Cell Line and Patient-Derived Xenografts. *J Mammary Gland Biol Neoplasia* **27**, 211-230, doi:10.1007/s10911-022-09520-y (2022).

194 Chuprin, J. *et al.* Humanized mouse models for immuno-oncology research. *Nat Rev Clin Oncol* **20**, 192-206, doi:10.1038/s41571-022-00721-2 (2023).

195 Deng, N. *et al.* Deep whole genome sequencing identifies recurrent genomic alterations in commonly used breast cancer cell lines and patient-derived xenograft models. *Breast Cancer Res* **24**, 63, doi:10.1186/s13058-022-01540-0 (2022).

196 Guiu, S. *et al.* Invasive lobular breast cancer and its variants: How special are they for systemic therapy decisions? *Critical Reviews in Oncology/Hematology* **92**, 235-257, doi:10.1016/j.critrevonc.2014.07.003 (2014).

197 Sikora, M. J. *et al.* Invasive Lobular Carcinoma Cell Lines Are Characterized by Unique Estrogen-Mediated Gene Expression Patterns and Altered Tamoxifen Response. *Cancer Research* **74**, 1463-1474, doi:10.1158/0008-5472.CAN-13-2779 (2014).

198 Vargo-Gogola, T. & Rosen, J. M. Modelling breast cancer: one size does not fit all. *Nat Rev Cancer* **7**, 659-672, doi:10.1038/nrc2193 (2007).

199 Zhang, X. *et al.* A Renewable Tissue Resource of Phenotypically Stable, Biologically and Ethnically Diverse, Patient-Derived Human Breast Cancer Xenograft Models. *Cancer Research* **73**, 4885-4897, doi:10.1158/0008-5472.CAN-12-4081 (2013).

200 Sflomos, G. *et al.* A Preclinical Model for ERα-Positive Breast Cancer Points to the Epithelial Microenvironment as Determinant of Luminal Phenotype and Hormone Response. *Cancer Cell* **29**, 407-422, doi:10.1016/j.ccell.2016.02.002 (2016).

201 Figueroa, J. D. *et al.* Terminal Duct Lobular Unit Involution of the Normal Breast: Implications for Breast Cancer Etiology. *JNCI: Journal of the National Cancer Institute* **106**, doi:10.1093/jnci/dju286 (2014).

202 DeRose, Y. S. *et al.* Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. *Nat Med* **17**, 1514-1520, doi:10.1038/nm.2454 (2011).

203 Behbod, F. *et al.* An intraductal human-in-mouse transplantation model mimics the subtypes of ductal carcinoma in situ. *Breast Cancer Res* **11**, R66, doi:10.1186/bcr2358 (2009).

204 Lutz, C. *et al.* Large-Scale Characterization of Orthotopic Cell Line-Derived Xenografts Identifies TGF-β Signaling as a Key Regulator of Breast Cancer Morphology and Aggressiveness. *Cancer Res*, doi:10.1158/0008-5472.Can-24-2022 (2025).

205 Woo, X. Y. *et al.* Conservation of copy number profiles during engraftment and passaging of patient-derived cancer xenografts. *Nat Genet* **53**, 86-99, doi:10.1038/s41588-020-00750-6 (2021).

206 Kabos, P. *et al.* Patient-derived luminal breast cancer xenografts retain hormone receptor heterogeneity and help define unique estrogen-dependent gene signatures. *Breast Cancer Res Treat* **135**, 415-432, doi:10.1007/s10549-012-2164-8 (2012).

207 Gao, H. *et al.* High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. *Nat Med* **21**, 1318-1325, doi:10.1038/nm.3954 (2015).

208 Özdemir, B. C., Sflomos, G. & Brisken, C. The challenges of modeling hormone receptor-positive breast cancer in mice. *Endocrine-Related Cancer* **25**, R319-R330, doi:10.1530/ERC-18-0063 (2018).

209 Clarke, R., Jones, B. C., Sevigny, C. M., Hilakivi-Clarke, L. A. & Sengupta, S. Experimental models of endocrine responsive breast cancer: strengths, limitations, and use. *CDR*, doi:10.20517/cdr.2021.33 (2021).

210 Cottu, P. *et al.* Modeling of response to endocrine therapy in a panel of human luminal breast cancer xenografts. *Breast Cancer Res Treat* **133**, 595-606, doi:10.1007/s10549-011-1815-5 (2012).

211 Behbod, F., Gomes, A. M. & Machado, H. L. Modeling Human Ductal Carcinoma In Situ in the Mouse. *J Mammary Gland Biol Neoplasia* **23**, 269-278, doi:10.1007/s10911-018-9408-0 (2018).

212 Hutten, S. J. *et al.* A living biobank of patient-derived ductal carcinoma in situ mouse-intraductal xenografts identifies risk factors for invasive progression. *Cancer Cell* **41**, 986-1002.e1009, doi:10.1016/j.ccell.2023.04.002 (2023).

213 Brodie, A. *et al.* Therapeutic observations in MCF-7 aromatase xenografts. *Clin Cancer Res* **11**, 884s-888s (2005).

214 Holliday, D. L. & Speirs, V. Choosing the right cell line for breast cancer research. *Breast Cancer Res* **13**, 215, doi:10.1186/bcr2889 (2011).

215 Band, V., Mirza, S., Mohibi, S. & Band, H. Mouse models of estrogen receptor-positive breast cancer. *J Carcinog* **10**, 35, doi:10.4103/1477-3163.91116 (2011).

216 Dabydeen, S. A. & Furth, P. A. Genetically engineered ERα-positive breast cancer mouse models. *Endocrine-Related Cancer* **21**, R195-R208, doi:10.1530/ERC-13-0512 (2014).

217 Kirma, N. B. & Tekmal, R. R. Transgenic mouse models of hormonal mammary carcinogenesis: Advantages and limitations. *The Journal of Steroid Biochemistry and Molecular Biology* **131**, 76-82, doi:10.1016/j.jsbmb.2011.11.005 (2012).

218 Miermont, A. M., Parrish, A. R. & Furth, P. A. Role of ER in the differential response of Stat5a loss in susceptibility to mammary preneoplasia and DMBA-induced carcinogenesis. *Carcinogenesis* **31**, 1124-1131, doi:10.1093/carcin/bgq048 (2010).

219 Tilli, M. T. *et al.* Introduction of Estrogen Receptor-α into the tTA/TAg Conditional Mouse Model Precipitates the Development of Estrogen-Responsive Mammary Adenocarcinoma. *The American Journal of Pathology* **163**, 1713-1719, doi:10.1016/S0002-9440(10)63529-8 (2003).

220 Green, J. E. *et al.* The C3(1)/SV40 T-antigen transgenic mouse model of mammary cancer: ductal epithelial cell targeting with multistage progression to carcinoma. *Oncogene* **19**, 1020-1027, doi:10.1038/sj.onc.1203280 (2000).

221 Rose-Hellekant, T. A. *et al.* Prolactin induces ERα-positive and ERα-negative mammary cancer in transgenic mice. *Oncogene* **22**, 4664-4674, doi:10.1038/sj.onc.1206619 (2003).

222 Rose-Hellekant, T. A. *et al.* Estrogen receptor-positive mammary tumorigenesis in TGFα transgenic mice progresses with progesterone receptor loss. *Oncogene* **26**, 5238-5246, doi:10.1038/sj.onc.1210340 (2007).

223 Zhang, X. *et al.* Estrogen receptor positivity in mammary tumors of Wnt-1 transgenic mice is influenced by collaborating oncogenic mutations. *Oncogene* **24**, 4220-4231, doi:10.1038/sj.onc.1208597 (2005).

224 Stratikopoulos, E. E. *et al.* Mouse ER+/PIK3CAH1047R breast cancers caused by exogenous estrogen are heterogeneously dependent on estrogen and undergo BIM-dependent apoptosis with BH3 and PI3K agents. *Oncogene* **38**, 47-59, doi:10.1038/s41388-018-0436-4 (2019).

225 Chan, S. R. *et al.* STAT1-deficient mice spontaneously develop estrogen receptor α-positive luminal mammary carcinomas. *Breast Cancer Res* **14**, R16, doi:10.1186/bcr3100 (2012).

226 Andò, S. *et al.* Conditional expression of Ki-RasG12V in the mammary epithelium of transgenic mice induces estrogen receptor alpha (ERα)-positive adenocarcinoma. *Oncogene* **36**, 6420-6431, doi:10.1038/onc.2017.252 (2017).

227 Campbell, K. M. *et al.* A Spontaneous Aggressive ERα+ Mammary Tumor Model Is Driven by Kras Activation. *Cell Reports* **28**, 1526-1537.e1524, doi:10.1016/j.celrep.2019.06.098 (2019).

228 Pearson, A. *et al.* Inactivating NF1 Mutations Are Enriched in Advanced Breast Cancer and Contribute to Endocrine Therapy Resistance. *Clinical Cancer Research* **26**, 608-622, doi:10.1158/1078-0432.CCR-18-4044 (2020).

229 Lima, A. & Maddalo, D. SEMMs: Somatically Engineered Mouse Models. A New Tool for In Vivo Disease Modeling for Basic and Translational Research. *Front Oncol* **11**, 667189, doi:10.3389/fonc.2021.667189 (2021).

230 Bu, W. & Li, Y. Advances in Immunocompetent Mouse and Rat Models. *Cold Spring Harb Perspect Med* **14**, a041328, doi:10.1101/cshperspect.a041328 (2024).

231 Tong, C., Huang, G., Ashton, C., Li, P. & Ying, Q.-L. Generating gene knockout rats by homologous recombination in embryonic stem cells. *Nat Protoc* **6**, 827-844, doi:10.1038/nprot.2011.338 (2011).

232 Chapman, Karen M. *et al.* Targeted Germline Modifications in Rats Using CRISPR/Cas9 and Spermatogonial Stem Cells. *Cell Reports* **10**, 1828-1835, doi:10.1016/j.celrep.2015.02.040 (2015).

233 Kim, K., Lindstrom, M. J. & Gould, M. N. Regions of H- and K-ras that provide organ specificity/potency in mammary cancer induction. *Cancer Res* **62**, 1241-1245 (2002).

234 Tai, Y.-T. & Gould, M. N. The genetic penetrance of the activated neu oncogene for the induction of mammary cancer in vivo. *Oncogene* **14**, 2701-2707, doi:10.1038/sj.onc.1201101 (1997).

235 Wang, B., Kennan, W. S., Yasukawa-Barnes, J., Lindstrom, M. J. & Gould, M. N. Frequent induction of mammary carcinomas following neu oncogene transfer into in situ mammary epithelial cells of susceptible and resistant rat strains. *Cancer Res* **51**, 5649-5654 (1991).

236 Bernardo, G. M. *et al.* FOXA1 is an essential determinant of ERα expression and mammary ductal morphogenesis. *Development* **137**, 2045-2054, doi:10.1242/dev.043299 (2010).

237 Cornelissen, L. M. *et al.* Exogenous ERα Expression in the Mammary Epithelium Decreases Over Time and Does Not Contribute to p53-Deficient Mammary Tumor Formation in Mice. *J Mammary Gland Biol Neoplasia* **24**, 305-321, doi:10.1007/s10911-019-09437-z (2019).

238 Rusidzé, M. *et al.* Estrogen receptor-α signaling in post-natal mammary development and breast cancers. *Cell. Mol. Life Sci.* **78**, 5681-5705, doi:10.1007/s00018-021-03860-4 (2021).

239 Kinoshita, Y. *et al.* Similarity of GATA-3 Expression between Rat and Human Mammary Glands. *J Toxicol Pathol* **27**, 159-162, doi:10.1293/tox.2014-0008 (2014).

240 Ström, J. O., Theodorsson, A., Ingberg, E., Isaksson, I.-M. & Theodorsson, E. Ovariectomy and 17β-estradiol Replacement in Rats and Mice: A Visual Demonstration. *JoVE*, 4013, doi:10.3791/4013 (2012).

241 Brown-Grant, K., Exley, D. & Naftolin, F. PERIPHERAL PLASMA OESTRADIOL AND LUTEINIZING HORMONE CONCENTRATIONS DURING THE OESTROUS CYCLE OF THE RAT. *Journal of Endocrinology* **48**, 295-296, doi:10.1677/joe.0.0480295 (1970).

242 Cardiff, R. D. & Wellings, S. R. The comparative pathology of human and mouse mammary glands. *J Mammary Gland Biol Neoplasia* **4**, 105-122, doi:10.1023/a:1018712905244 (1999).

243 Nicotra, R., Lutz, C., Messal, H. A. & Jonkers, J. Rat Models of Hormone Receptor-Positive Breast Cancer. *J Mammary Gland Biol Neoplasia* **29**, 12, doi:10.1007/s10911-024-09566-0 (2024).

## **Supporting Table 1:** Human breast cancer cell lines

| Cell line | DepMap (Broad) | Cancer Cell Line Encyclopedia (CCLE) | Cellosaurus | Cell model passport (Sanger Model ID) | COSMIC (Sanger) | Primary Disease | Tumour type | Primary/ Metastasis | Collection Site | Molecular subtype (DepMap) | Patient Age | Patient Gender |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 21MT1 | ACH-002400 | n.d. | CVCL_7931 | SIDM01902 | n.d. | Breast Neoplasm, NOS | Breast Neoplasm, NOS (BNNOS) | Metastatic | Pleural effusion | n.d. | 37 | female |
| 21MT2 | ACH-002401 | 21MT2_BREAST | CVCL_7932 | SIDM01901 | n.d. | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Metastatic | pleural_effusion | n.d. | 37 | female |
| 21NT | ACH-002399 | 21NT_BREAST | CVCL_7933 | SIDM01912 | n.d. | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | n.d. | 36 | female |
| 21PT | n.d. | n.d. | CVCL_7934 | SIDM01900 | n.d. | Breast Ductal Carcinoma In Situ | Breast Ductal Carcinoma In Situ (DCIS) | Primary | breast | n.d. | 36 | female |
| 600MPE | ACH-002320 | 600MPE_BREAST | CVCL_9875 | n.d. | 1000128 | Invasive Breast Carcinoma | Breast Invasive Carcinoma, NOS (BRCNOS) | Metastatic | pleural_effusion | n.d. | 46 | female |
| AU565 | ACH-000248 | n.d. | CVCL_1074 | SIDM00898 | 910704 | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Metastatic | pleural_effusion | HER2_amp | 43 | female |
| BCK4 | ACH-002921 | n.d. | CVCL_A9A5 | n.d. | n.d. | Invasive Breast Carcinoma | Breast Invasive Lobular Carcinoma (ILC) | Metastatic | pleural_effusion | n.d. | 65 | female |
| BT20 | ACH-000536 | BT20_BREAST | CVCL_0178 | SIDM00893 | 906801 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | basal_A | 74 | female |
| BT474 | ACH-000927 | BT474_BREAST | CVCL_0179 | SIDM00963 | 946359 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | HER2_amp | 60 | female |
| BT483 | ACH-000818 | BT483_BREAST | CVCL_2319 | SIDM00892 | 949093 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | luminal | 23 | female |
| BT549 | ACH-000288 | BT549_BREAST | CVCL_1092 | SIDM00122 | 905951 | Invasive Breast Carcinoma | Breast Invasive Carcinoma, NOS (BRCNOS) | Primary | breast | basal_B | 72 | female |
| CAL120 | ACH-000212 | CAL120_BREAST | CVCL_1104 | SIDM00940 | 906826 | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Metastatic | pleural_effusion | basal_B | 43 | female |
| CAL148 | ACH-000902 | CAL148_BREAST | CVCL_1106 | SIDM00938 | 924106 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Metastatic | pleural_effusion | luminal_HER2_amp | 58 | female |
| CAL51 | ACH-000856 | CAL51_BREAST | CVCL_1110 | SIDM00933 | 910927 | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Metastatic | pleural_effusion | basal_B | 45 | female |
| CAL851 | ACH-000857 | CAL851_BREAST | CVCL_1114 | SIDM00928 | 910852 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | basal_A | 35 | female |
| CAMA1 | ACH-000783 | CAMA1_BREAST | CVCL_1115 | SIDM00920 | 946382 | Invasive Breast Carcinoma | Breast Invasive Lobular Carcinoma (ILC) | Metastatic | pleural_effusion | luminal | 51 | female |
| COLO824 | ACH-001820 | COLO824_BREAST | CVCL_1136 | SIDM00954 | n.d. | Breast Neoplasm, NOS | Breast Neoplasm, NOS (BNNOS) | Metastatic | pleural_effusion | n.d. | 52 | female |
| DU4475 | ACH-000258 | DU4475_BREAST | CVCL_1183 | SIDM01001 | 906844 | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Metastatic | skin | luminal_HER2_amp | 62 | female |
| EFM19 | ACH-000330 | EFM19_BREAST | CVCL_0253 | SIDM01056 | 906851 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Metastatic | pleural_effusion | luminal | 50 | female |
| EFM192A | ACH-000117 | EFM192A_BREAST | CVCL_1812 | SIDM01002 | 1290798 | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Metastatic | pleural_effusion | HER2_amp | 46 | female |
| EFM192B | n.d. | n.d. | CVCL_1813 | SIDM01054 | n.d. | Breast Neoplasm, NOS | Breast Neoplasm, NOS (BNNOS) | Metastatic | pleural_effusion | n.d. | 46 | female |
| EFM192C | n.d. | n.d. | CVCL_1814 | SIDM01053 | n.d. | Breast Neoplasm, NOS | Breast Neoplasm, NOS (BNNOS) | Metastatic | pleural_effusion | n.d. | 46 | female |
| EVSA-T | ACH-001065 | EVSAT_BREAST | CVCL_1207 | SIDM01042 | 906862 | Invasive Breast Carcinoma | Breast Invasive Lobular Carcinoma (ILC) | Metastatic | ascites | n.d. | 58 | female |
| HCC1008 | ACH-001514 | HCC1008_BREAST | CVCL_1244 | n.d. | 1235076 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Metastatic | lymph_node | n.d. | 67 | female |
| HCC1143 | ACH-000374 | HCC1143_BREAST | CVCL_1245 | SIDM00866 | 749710 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | basal_A | 52 | female |
| HCC1187 | ACH-000111 | HCC1187_BREAST | CVCL_1247 | SIDM00885 | 749711 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | basal_A | 41 | female |
| HCC1395 | ACH-000699 | HCC1395_BREAST | CVCL_1249 | SIDM00884 | 749712 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | basal_B | 43 | female |
| HCC1419 | ACH-000277 | HCC1419_BREAST | CVCL_1251 | SIDM00882 | 907045 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | HER2_amp | 42 | female |
| HCC1428 | ACH-000352 | HCC1428_BREAST | CVCL_1252 | SIDM00881 | 1290905 | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Metastatic | pleural_effusion | luminal | 49 | female |
| HCC1500 | ACH-000349 | HCC1500_BREAST | CVCL_1254 | SIDM00879 | 1303900 | Breast Ductal Carcinoma In Situ | Breast Ductal Carcinoma In Situ (DCIS) | Primary | breast | luminal | 32 | female |
| HCC1569 | ACH-000930 | HCC1569_BREAST | CVCL_1255 | SIDM00878 | 907046 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | basal_A | 70 | female |
| HCC1599 | ACH-000196 | HCC1599_BREAST | CVCL_1256 | SIDM00877 | 749713 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | basal_A | 44 | female |
| HCC1806 | ACH-000624 | HCC1806_BREAST | CVCL_1258 | SIDM00875 | 907047 | Breast Ductal Carcinoma In Situ | Breast Ductal Carcinoma In Situ (DCIS) | Primary | breast | basal_A | 60 | female |
| HCC1937 | ACH-000223 | HCC1937_BREAST | CVCL_0290 | SIDM00874 | 749714 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | basal_A | 24 | female |

| Cell line | DepMap (Broad) | Cancer Cell Line Encyclopedia (CCLE) | Cellosaurus | Cell model passport (Sanger Model ID) | COSMIC (Sanger) | Primary Disease | Tumour type | Primary/ Metastasis | Collection Site | Molecular subtype (DepMap) | Patient Age | Patient Gender |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HCC1954 | ACH-000859 | HCC1954_BREAST | CVCL_1259 | SIDM00872 | 749709 | Breast Ductal Carcinoma In Situ | Breast Ductal Carcinoma In Situ (DCIS) | Primary | breast | basal_A | 61 | female |
| HCC202 | ACH-000725 | HCC202_BREAST | CVCL_2062 | SIDM00870 | 1290906 | Breast Ductal Carcinoma In Situ | Breast Ductal Carcinoma In Situ (DCIS) | Primary | breast | HER2_amp | 82 | female |
| HCC2157 | ACH-000691 | HCC2157_BREAST | CVCL_1261 | SIDM00774 | 749715 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | basal_A | 48 | female |
| HCC2185 | ACH-002322 | HCC2185_BREAST | CVCL_3375 | n.d. | 1136368 | Invasive Breast Carcinoma | Breast Invasive Lobular Carcinoma (ILC) | Metastatic | pleural_effusion | n.d. | 49 | female |
| HCC2218 | ACH-000755 | HCC2218_BREAST | CVCL_1263 | SIDM00772 | 749716 | Breast Ductal Carcinoma In Situ | Breast Ductal Carcinoma In Situ (DCIS) | Primary | breast | HER2_amp | 38 | female |
| HCC2688 | ACH-002323 | HCC2688_BREAST | CVCL_3376 | n.d. | n.d. | Breast Ductal Carcinoma In Situ | Breast Ductal Carcinoma In Situ (DCIS) | n.d. | breast | n.d. | n.d. | female |
| HCC3153 | ACH-002324 | HCC3153_BREAST | CVCL_3377 | n.d. | 1136375 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | n.d. | breast | n.d. | n.d. | female |
| HCC38 | ACH-000276 | HCC38_BREAST | CVCL_1267 | SIDM00675 | 749717 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | basal_A | 50 | female |
| HCC70 | ACH-000668 | HCC70_BREAST | CVCL_1270 | SIDM00673 | 907048 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | basal_A | 49 | female |
| HDQ-P1 | ACH-000643 | HDQP1_BREAST | CVCL_2067 | SIDM01062 | 1290922 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | basal_A | 50 | female |
| HMC1-8 | ACH-000721 | HMC18_BREAST | CVCL_2949 | SIDM01622 | n.d. | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Metastatic | pleural_effusion | basal_B | 35 | female |
| Hs578T | ACH-000148 | HS578T_BREAST | CVCL_0332 | SIDM00135 | 905957 | Invasive Breast Carcinoma | Breast Invasive Carcinoma, NOS (BRCNOS) | Primary | breast | basal_B | 74 | female |
| IPMBO053 | ACH-002883 | n.d. | n.d. | n.d. | n.d. | Invasive Breast Carcinoma | Breast Invasive Lobular Carcinoma (ILC) | Metastatic | breast | n.d. | 77 | female |
| IPMBO055 | ACH-002884 | n.d. | n.d. | n.d. | n.d. | Invasive Breast Carcinoma | Breast Invasive Lobular Carcinoma (ILC) | Metastatic | breast | n.d. | 77 | female |
| IPMBO056 | ACH-002885 | n.d. | n.d. | n.d. | n.d. | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | n.d. | 43 | female |
| JIMT1 | ACH-000711 | JIMT1_BREAST | CVCL_2077 | SIDM01037 | 1298157 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Metastatic | pleural_effusion | basal_A | 62 | female |
| KPL1 | ACH-000028 | KPL1_BREAST | CVCL_2094 | SIDM00147 | n.d. | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Metastatic | pleural_effusion | luminal | 69 | female |
| LY2 | ACH-002325 | LY2_BREAST | CVCL_9579 | n.d. | n.d. | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Metastatic | breast | n.d. | 69 | female |
| MACLS2 | ACH-002326 | MACLS2_BREAST | CVCL_4571 | n.d. | n.d. | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Metastatic | pleural_effusion | n.d. | 47 | female |
| MCF7 | ACH-000019 | MCF7_BREAST | CVCL_0031 | SIDM00148 | 905946 | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Metastatic | pleural_effusion | luminal | 69 | female |
| MDA-MB-134-VI | ACH-000044 | MDAMB134VI_ BREAST | CVCL_0617 | SIDM00005 | 1289392 | Invasive Breast Carcinoma | Breast Invasive Lobular Carcinoma (ILC) | Metastatic | pleural_effusion | luminal | 47 | female |
| MDA-MB-157 | ACH-000621 | MDAMB157_BREAST | CVCL_0618 | SIDM00529 | 925338 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Metastatic | pleural_effusion | basal_B | 44 | female |
| MDA-MB-175-VII | ACH-000759 | MDAMB175VII_ BREAST | CVCL_1400 | SIDM00633 | 908120 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Metastatic | pleural_effusion | HER2_amp | 56 | female |
| MDA-MB-231 | ACH-000768 | MDAMB231_BREAST | CVCL_0062 | SIDM00146 | 905960 | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Metastatic | pleural_effusion | basal_B | 51 | female |
| MDA-MB-330 | ACH-001358 | MDAMB330_BREAST | CVCL_0619 | SIDM00631 | 1330941 | Invasive Breast Carcinoma | Breast Invasive Lobular Carcinoma (ILC) | Metastatic | pleural_effusion | n.d. | 43 | female |
| MDA-MB-361 | ACH-000934 | MDAMB361_BREAST | CVCL_0620 | SIDM00528 | 908121 | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Metastatic | central_nervous_ system | HER2_amp | 40 | female |
| MDA-MB-415 | ACH-000876 | MDAMB415_BREAST | CVCL_0621 | SIDM00630 | 924240 | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Metastatic | pleural_effusion | HER2_amp | 38 | female |
| MDA-MB-436 | ACH-000573 | MDAMB436_BREAST | CVCL_0623 | SIDM00629 | 1240172 | Invasive Breast Carcinoma | Breast Invasive Carcinoma, NOS (BRCNOS) | Metastatic | pleural_effusion | basal_B | 43 | female |
| MDA-MB-453 | ACH-000910 | MDAMB453_BREAST | CVCL_0418 | SIDM00272 | 908122 | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Metastatic | pericardial_ effusion | HER2_amp | 48 | female |
| MDA-MB-468 | ACH-000849 | MDAMB468_BREAST | CVCL_0419 | SIDM00628 | 908123 | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Metastatic | pleural_effusion | basal_A | 51 | female |
| MFM223 | ACH-001819 | MFM223_BREAST | CVCL_1408 | SIDM00332 | 2165000 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Metastatic | pleural_effusion | luminal | n.d. | female |
| MRKnu1 | ACH-002163 | MRKNU1_BREAST | CVCL_1428 | SIDM00562 | 908151 | Invasive Breast Carcinoma | Breast Invasive Lobular Carcinoma (ILC) | Primary | breast | n.d. | 46 | female |

| Cell line | DepMap (Broad) | Cancer Cell Line Encyclopedia (CCLE) | Cellosaurus | Cell model passport (Sanger Model ID) | COSMIC (Sanger) | Primary Disease | Tumour type | Primary/ Metastasis | Collection Site | Molecular subtype (DepMap) | Patient Age | Patient Gender |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MX1 | ACH-002328 | MX1_BREAST | CVCL_4774 | n.d. | 398600 | Breast Neoplasm, NOS | Breast Neoplasm, NOS (BNNOS) | n.d. | breast | n.d. | 29 | female |
| NH84T | ACH-002951 | n.d. | n.d. | n.d. | n.d. | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Primary | breast | n.d. | n.d. | female |
| NH93T | ACH-002950 | n.d. | n.d. | n.d. | n.d. | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Primary | breast | n.d. | n.d. | female |
| OCUB-M | ACH-002179 | OCUBM_BREAST | CVCL_1621 | SIDM00241 | 909256 | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Metastatic | pleural_effusion | n.d. | 53 | female |
| SKBR3 | ACH-000017 | SKBR3_BREAST | CVCL_0033 | SIDM00897 | 871159 | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Metastatic | pleural_effusion | HER2_amp | 43 | female |
| SKBR5 | ACH-002329 | SKBR5_BREAST | CVCL_5217 | n.d. | n.d. | Breast Neoplasm, NOS | Breast Neoplasm, NOS (BNNOS) | n.d. | breast | n.d. | n.d. | female |
| SKBR7 | ACH-002330 | SKBR7_BREAST | CVCL_5218 | n.d. | n.d. | Breast Neoplasm, NOS | Breast Neoplasm, NOS (BNNOS) | n.d. | breast | n.d. | n.d. | female |
| SNU2372 | ACH-001662 | SNU2372_BREAST | CVCL_T041 | n.d. | n.d. | Invasive Breast Carcinoma | Breast Invasive Cancer, NOS (BRCANOS) | Primary | pleural_effusion | n.d. | 55 | female |
| SUM102PT | ACH-001388 | SUM102PT_BREAST | CVCL_3421 | SIDM01837 | 1289410 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | basal | 57 | female |
| SUM1315MO2 | ACH-001389 | SUM1315MO2_ BREAST | CVCL_5589 | SIDM01836 | 1289417 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Metastatic | skin | basal | n.d. | female |
| SUM149PT | ACH-001390 | SUM149PT_BREAST | CVCL_3422 | SIDM01441 | 1289411 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | basal_B | 40 | female |
| SUM159PT | ACH-001391 | SUM159PT_BREAST | CVCL_5423 | SIDM01452 | 1289412 | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Primary | breast | basal_B | 71 | female |
| SUM185PE | ACH-001392 | SUM185PE_BREAST | CVCL_5591 | SIDM01430 | 1289413 | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Metastatic | pleural_effusion | luminal | n.d. | female |
| SUM190PT | ACH-001393 | SUM190PT_BREAST | CVCL_3423 | n.d. | 1289414 | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Primary | breast | basal_A | n.d. | female |
| SUM225CWN | ACH-001397 | SUM225CWN_ BREAST | CVCL_5593 | n.d. | 1289415 | Invasive Breast Carcinoma | Breast Invasive Cancer, NOS (BRCANOS) | Metastatic | breast | basal | 56 | female |
| SUM229PE | ACH-001394 | SUM229PE_BREAST | CVCL_5594 | SIDM01461 | 1289416 | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Metastatic | pleural_effusion | basal | n.d. | female |
| SUM44PE | ACH-001395 | SUM44PE_BREAST | CVCL_3424 | SIDM01835 | 1289408 | Invasive Breast Carcinoma | Breast Invasive Lobular Carcinoma (ILC) | Metastatic | pleural_effusion | luminal | n.d. | female |
| SUM52PE | ACH-001396 | SUM52PE_BREAST | CVCL_3425 | SIDM01422 | 1289409 | Invasive Breast Carcinoma | Invasive Breast Carcinoma (BRCA) | Metastatic | pleural_effusion | luminal | n.d. | female |
| T47D | ACH-000147 | T47D_BREAST | CVCL_0553 | SIDM00097 | 905945 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Metastatic | pleural_effusion | luminal | 54 | female |
| UACC3133 | ACH-001683 | UACC3133_BREAST | CVCL_B221 | n.d. | n.d. | Invasive Breast Carcinoma | Breast Invasive Lobular Carcinoma (ILC) | Primary | breast | n.d. | 52 | female |
| UACC-812 | ACH-000568 | UACC812_BREAST | CVCL_1781 | SIDM01187 | 910910 | Invasive Breast Carcinoma | Breast Invasive Lobular Carcinoma (ILC) | Primary | breast | luminal | 43 | female |
| UACC-893 | ACH-000554 | UACC893_BREAST | CVCL_1782 | SIDM01186 | 909778 | Invasive Breast Carcinoma | Breast Invasive Lobular Carcinoma (ILC) | Primary | breast | HER2_amp | 57 | female |
| VP229 | ACH-001419 | VP229_BREAST | CVCL_2754 | SIDM01826 | 1523834 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | n.d. | 47 | female |
| VP267 | ACH-001705 | VP267_BREAST | CVCL_2755 | SIDM01825 | n.d. | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Primary | breast | n.d. | 48 | female |
| YMB-1 | ACH-001249 | YMB1_BREAST | CVCL_2814 | SIDM01260 | n.d. | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Metastatic | ascites | luminal | 63 | female |
| YMB-1-E | ACH-002208 | YMB1E_BREAST | CVCL_2815 | SIDM01261 | 1303911 | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Metastatic | ascites | luminal | 63 | female |
| ZR75-1 | ACH-000097 | ZR751_BREAST | CVCL_0588 | SIDM00314 | n.d. | Invasive Breast Carcinoma | Breast Invasive Ductal Carcinoma (IDC) | Metastatic | ascites | luminal | 63 | female |
| ZR75-30 | ACH-000828 | ZR7530_BREAST | CVCL_1661 | SIDM00971 | 909907 | Invasive Breast Carcinoma | Breast Invasive Lobular Carcinoma (ILC) | Metastatic | ascites | HER2_amp | 47 | female |
| ZR75B | ACH-002331 | ZR75B_BREAST | CVCL_5614 | n.d. | n.d. | Invasive Breast Carcinoma | Breast Invasive Carcinoma, NOS (BRCNOS) | Metastatic | ascites | n.d. | 63 | female |