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Genetically Engineered ERα positive breast cancer mouse models

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Abstract

The majority of human breast cancers are ER+ but this has proven challenging to model in genetically engineered mice. This review summarizes information on twenty-one mouse models that develop ER+ mammary cancer. Where available, information on cancer pathology and gene expression profiles is referenced to assist in understanding which histological subtype of ER+ human cancer each model might represent. *Esr1*, *Ccdn1*, *prolactin*, *TGF*α, AIB1, *Espl1*, and *Wnt1* over-expression, *Pik3ca* gain of function, as well as loss of *p53* or loss of *Stat1* are associated with ER+ mammary cancer. Treatment with the PPARγ agonist efatutazone in a mouse with *Brca1* and *p53* deficiency and DMBA exposure in combination with an activated myristoylated form of AKT1 also induce ER+ mammary cancer. A spontaneous mutant in nude mice that develops metastatic ER+ mammary cancer is included. Age of cancer development ranges from three to 26 months and the percentages of cancers that are ER+ vary from 21% to 100%. Not all models are characterized as to their estrogen dependency and/or response to anti-hormonal therapy. Strain backgrounds include C57Bl/6, FVB, BALB/c, 129S6/SvEv, CB6F1 and NIH nude. Most models have only been studied on one strain background. In summary while a range of models is available for studies of pathogenesis and therapy of ER+ breast cancers, many could benefit from further characterization and opportunity for development of new models remains.

Keywords

mammary gland; estrogen receptor; breast; carcinoma; pathogenesis

Introduction

Breast cancer is a heterogeneous disease consisting of four clinically relevant categories based on expression patterns of estrogen receptor alpha (ERα; ESR1) and V-Erb-B2 Avian Erythroblastic Leukemia Viral Oncogene Homolog 2 (HER2) (Guiu, et al. 2012). Molecular

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studies divide breast cancers expressing ERα into luminal A and luminal B sub-types that are distinguished by different expression patterns of proliferation-related genes. Together they are referred to as ER+ breast cancers. But within this classification exists different morphological/histological sub-types (Habashy, et al. 2012). The majority of human invasive ER+ breast cancers are classified as invasive ductal, smaller percentages are defined as invasive lobular, while other histological subtypes including tubular, papillary, invasive cribriform, mucinous, adenocystic, adenosquamous, spindloid, adenomyoepithelioma appear less commonly. Ductal carcinoma in situ (DCIS) is a non-invasive cancer. ER+ breast cancer may or may not express progesterone receptor (PGR) and/or HER2+. Triple negative breast cancer does not express ER, PGR or enriched HER2 and includes basal-like breast cancer. ER+ luminal subtypes represent approximately 70% of all invasive breast cancers diagnosed in the United States each year (Yanagawa, et al. 2012).

ER+ breast cancer is defined if one to ten percent of the cancer cell nuclei stain for ERα by immunohistochemistry (IHC) (Hammond, et al. 2010; Harvey, et al. 1999). Women diagnosed with ER+ breast cancer are candidates for anti-hormonal therapy including tamoxifen, raloxifene, fulvestrant and the aromatase inhibitors letrozole, exemestane, vorozole, formestane, and fadrozole (Ariazi, et al. 2006; Geisler, et al. 2012; Larionov and Miller 2009).

Classically, ERα is activated by binding to estrogens resulting in nuclear translocation with binding to estrogen response elements (ERE) and expression of estrogen target genes as a major mechanism of action. Membrane/cytoplasmic and G-protein-coupled activities are also described (Renoir, et al. 2013). ERβ, which is encoded by a different gene, is generally characterized as being anti-proliferative in breast cancer (Fox, et al. 2008). A wide range of molecular activities are known to influence expression levels and activity of ERα with many of these pathways having the capacity to contribute to breast cancer development (Manavathi, et al. 2013). In both women (Allred, et al. 2004) and mice (Frech, et al. 2005) deregulated estrogen signaling can result in increased proliferation of the mammary ductal epithelium leading to cancer progression.

Creation of immortalized ER+ breast cancer cell lines was an important step for the experimental study of ER+ breast cancer pathogenesis and therapy *in vitro* (Holliday and Speirs 2011; Wong and Chen 2012). Application of these cell lines to xenograft models has enabled a wide variety of *in vivo* studies examining response to therapy including anti-hormonal approaches (Brodie, et al. 2005). Norway rats exposed to chemical carcinogens develop ER+ mammary cancer and have been used in different types of *in vivo* experiments exploring pathogenesis and treatment (Shull 2007).

Genetic engineering of mouse models to produce ER+ mammary cancer represents an alternative choice for *in* vivo study of ER+ breast cancer pathogenesis and treatment. Mice are more readily genetically manipulated than rats, there are many genetically engineered mouse models to breed into ER+ breast cancer models for further study, and mice are, in general, less expensive to maintain than rats. Mouse models of estrogen receptor-positive breast cancer have been reviewed previously (Kirma and Tekmal 2012; Mohibi, et al. 2011). Here we update the discussion with more recently developed models as well as include new

complementary information on those that have been described before. To date five major types of models have been published (Table 1). The first type develops ER+ mammary cancer from direct over-expression of ERα in mammary epithelial cells (Table 1A). The second type exhibits ER+ mammary cancer as a result of genetic aberrations of other molecules within the estrogen-signaling pathway (Table 1B). The third type develops ER+ mammary cancer as a result of pharmacologic treatment in combination with genetic aberrations of other molecules within the estrogen-signaling pathway (Table 1C). The fourth type results from exposure to a chemical carcinogen in combination with genetic aberrations of other molecules within the estrogen-signaling pathway (Table 1D). The fifth type is derived from brother-sister matings of nude mice (Table 1E).

At the present time it is clear that there in no one mouse model that develops all of the histopathological types or molecular sub-types of ER+ human breast cancer (Malhotra, et al. 2010). Human breast cancers are classified as non-invasive or invasive and ER+ cancers are found in both categories. Invasive (also called infiltrating) ductal carcinoma is the most common type of human breast cancer and the majority of these are ER+. Histopathological types of human breast cancer that are even more commonly ER+ include invasive lobular, papillary and tubular. Not all of the published studies that report the development of genetically engineered mice provide sufficient detail to be able to definitively assign the histopathology developing in the mouse to the corresponding human histopathology. Similarly, not all models have been yet adequately molecularly analyzed to be able to accurately assign molecular sub-type. However, where this information is available, it is reported in the text as part of the description of the model

The transcriptomes of mouse and human mammary epithelial cells demonstrate significant similarities in gene and pathway activation (Lim, et al. 2010). However there are differences in ER expression patterns. Luminal progenitor cells in humans are reported to express higher levels of ER than luminal progenitor cells in mice (Visvader 2009). In both normal human and mouse mammary gland, ER is expressed in a portion of the non-proliferating luminal mammary epithelial cells whereas premalignant and malignant lesions demonstrate proliferating ER+ mammary cells (Anderson and Clarke 2004; Anderson, et al. 1998). However, whereas human mammary stroma does not demonstrate expression of ER, ER is expressed in the mouse mammary stroma where it is able to act in a paracrine fashion on the mammary epithelium (Parmar and Cunha 2004).

The genetically engineered mouse models presented here utilize the mouse mammary tumor virus (MMTV) long terminal repeat and the rat neu-related lipocalin (Nrl) promoter to drive coding sequences of cancer-inducing proteins. MMTV and the Whey Acidic Protein (WAP) promoter are used to drive expression of the cre recombinase. The MMTV and WAP promoter sequences contain hormone responsive elements while the Nrl promoter is hormonally non-responsive. Molecular studies *in vitro* demonstrate that while the MMTV promoter is responsive to glucocorticoid receptor, androgen receptor, and PGR, it is not an ER responsive promoter (Prefontaine, et al. 1999). In vivo, expression of ErbB2 from an MMTV promoter is not significantly increased by coincident expression of aromatase, which increases estrogen production (Tekmal, et al. 2007). The WAP-Cre transgene is active only during lactation while MMTV-Cre is active throughout mammary development

(Wagner, et al. 1997). Studies that perform ovariectomy to demonstrate estrogen responsiveness of MMTV driven cancers should take into account that ovariectomy will result in loss of both estrogen and progesterone, rendering the possibility that tumor regression could be confounded by a decrease in transgene expression levels due to the loss of progesterone. Appropriate controls to perform are to directly assess transgene expression in the presence and absence of ovariectomy. Similarly, if exogenous hormones including estrogen and progesterone are used, their impact on transgene expression levels should be characterized. The same transgene in different integration sites can exhibit different regulatory behavior so one cannot generalize about the hormone dependency of a specific transgene from one line to another (Wagner, et al. 2001). Finally some hormonally unresponsive promoters within transgenes can nevertheless demonstrate a dependency for expression on hormonally regulated developmental stages such as the C3(1)/T(AG) transgene, whose expression is turned on with puberty but does not demonstrate differences in expression in response to isolated alterations in estrogen or ER levels (Yoshidome, et al. 2000). Finally, hormonally responsive promoters can lose their dependence upon hormonal signals as reported for the *WAP-rtTACre* transgene by (Lin, et al. 2004).

Estrogen receptor 1 over-expression mouse models

The addition of murine *Esr1* expression to a mouse model in which expression of Simian Virus 40 T Antigen (TAg) is directed to epithelial tissues using a conditional tetracycline responsive gene expression system induces the appearance of ER+ mammary adenocarcinoma in 37% of female *tet-op-Esr1*[*Mouse Mammary Tumor Virus (MMTV)-tetracycline TransActivator (tTA)/tetracycline-operator (tet-op)-TAg)*] mice by 12 months of age (Tilli, et al. 2003) (Table 1A). All of the mammary cancers that appear in this model are ER+. The cancers have been shown to bind estrogen and demonstrate estrogen dependence. In this model expression of both *Esr1* and *Simian Virus 40 T Antigen* are targeted together to epithelial cells and temporally regulated using an *MMTV-tTA* ‘tet-off’ transgene (Furth 1997; Hennighausen, et al. 1995). A ‘tet-off’ regulated transgene is expressed in the absence of exposure of the mice to a tetracycline compound such as doxycycline. Administration of doxycycline to a transgenic mouse carrying the ‘tet-off’ system results in doxycycline binding to the tetracycline responsive transactivator protein, which changes its conformation and renders it unable to bind to the tetracycline responsive promoter. In contrast in the ‘tet-on’ system a mutated tetracycline responsive transactivator protein is expressed that binds a tetracycline responsive promoter only when it is bound to a tetracycline compound such as doxycycline. Both systems can be used equally as efficiently in transgenic mice to temporally direct transgene expression. Significantly, in the absence of coincident TAg expression, *Tet-op-Esr1*$^{MMTV\text{-}tTA}$ transgenic mice do not develop mammary cancer. The adenocarcinomas that develop in the *tet-op-Esr1*$^{MMTV\text{-}tTA/tet\text{-}op\text{-}SV40\text{-}TAg}$ mice histologically model human ER+ ductal adenocarcinoma.

In contrast, utilization of a different conditional transgene, (*MMTV*-rtTA) (Gunther, et al. 2002), to target murine *Esr1* over-expression to mammary epithelial cells results in three to five percent of the *Tet-op-Esr1*$^{MMTV\text{-}rtTA}$ mice exhibiting mammary adenocarcinoma by 12 months of age (Miermont, et al. 2012; Miermont, et al. 2010) (Table 1A). Half of the

invasive adenocarcinomas that appear are ERα+ and neither cancer prevalence nor percentage of ERα+ adenocarcinoma are altered by low-dose DMBA exposure, loss of the Stat5a gene or coincident cyclin D1 over-expression. Prevalence of preneoplasia is higher than that of cancer with 30% of the mice reproducibly demonstrating ductal hyperplasia (DH) and 40% hyperplastic alveolar nodules (HANs) by age 12 months (Diaz-Cruz, et al. 2011; Nakles, et al. 2011). In this model expression of ERα is increased 1.5 to 2-fold in mammary epithelial cells resulting in the appearance of increased mammary ductal epithelial cell proliferation and the appearance of DH and DCIS by four months of age (Frech et al. 2005). Disease appearance is dependent upon the presence of cyclin D1 (Frech, et al. 2008). Loss of one copy of *p53* significantly increases preneoplasia prevalence but not cancer (Diaz-Cruz and Furth 2010). Significantly ERα+ invasive adenocarcinomas have developed on tamoxifen in this model and tamoxifen delivered at 10 months of age fails to induce a significant reduction in HAN prevalence, consistent with the presence of a significant degree of intrinsic tamoxifen resistance (Miermont 2012). The impact of a 'tet-off' transgene (*tet-op-tTA*), whose expression is on an autoregulatory loop (Shockett, et al. 1995), to target *tet-op-Esr1* expression to both epithelial and non-epithelial tissues has also been investigated (Tomic, et al. 2007). Mammary ductal hyperplasia also appears in these mice by 4 months of age (P.A.F. unpublished data). The higher rates of preneoplasia and cancer development in *Tet-op-Esr1*$^{MMTV\text{-}rtTA}$ as compared to *Tet-op-Esr1*$^{MMTV\text{-}tTA}$ mice is correlated with a significantly higher percentage of mammary epithelial cells demonstrating targeted transgene expression (Gunther, et al. 2002; Hennighausen et al. 1995). The *tet-op-Esr1*$^{MMTV\text{-}rtTA}$ mice histologically model human DCIS and ductal adenocarcinoma. Adenosquamouscarcinomas, modeling a less common human sub-type, appear less frequently.

Amplified in Breast cancer 1 (AIB1), also known as steroid receptor coactivator 3 (SRC-3) or thyroid hormone receptor activator molecule 1 (TRAM-1), impacts the activity of both hormone-dependent and –independent pathways in breast cancer and has been proposed as a modulator of tamoxifen resistance (Lahusen, et al. 2009; Xu, et al. 2009). A splice variant lacking the N-terminal domain (AIB1Δ3/AIB1-Δ4) is a more potent inducer of estrogen-mediated gene transcription (Chien, et al. 2011). Tri-transgenic *tet-op-Esr1*$^{MMTV\text{-}tTA/tet\text{-}op\text{-}AIB1}$ and *tet-op-Esr1*$^{MMTV\text{-}tTA/tet\text{-}op\text{-}AIB1\Delta3}$ mice were generated to compare the impact of AIB1 and AIB1Δ3 on ERα-mediated mammary carcinogenesis (Nakles et al. 2011) (Table 1A). ER+ mammary adenocarcinomas modeling human invasive ductal carcinoma developed in the *tet-op-Esr1*$^{MMTV\text{-}tTA/tet\text{-}op\text{-}AIB1\Delta3}$ mice but the prevalence was not significantly different than that found in the *Tet-op-Esr1*$^{MMTV\text{-}rtTA}$ mice and cancers did not appear until 19–26 months of age, rendering no advantages of this genetic combination over the *Tet-op-Esr1*$^{MMTV\text{-}rtTA}$ mice for the study of ER+ mammary cancer.

Loss of the BReast CAncer 1, early-onset 1 (*BRCA1*) gene is a genetic risk factor for development of breast cancer. A high percentage of women born with deleterious mutations in the BRCA1 gene will develop breast cancer by age 70 (Chen and Parmigiani 2007). The predilection for cancer development in estrogen responsive tissues in women carrying BRCA1 mutations may be related to the ability of BRCA1 to down-regulate activity of ERα (Eisen, et al. 2008). *Tet-op-Esr1*$^{MMTV\text{-}rtTA}$ mice were mated to mice with genetically

engineered conditional deletion of exon 11 of the *Brca1* gene in mammary epithelial cells to generate *Tet-op-Esr1*$^{MMTV\text{-}rtTA}$/Brca1 floxed exon 11 $^{(f11)/f11/MMTV\text{-}Cre}$ for testing the impact of ERα overexpression on cancer development initiated by loss of BRCA1 function (Jones, et al. 2008). In the absence of *Esr1* over-expression, 53% of *Brca1* deficient mice (Brca1^{f11} $^{(f11)/f11/MMTV\text{-}Cre/p53+/-}$) develop triple negative (ER−/PGR−/HER2<2+) adenocarcinomas with gene expression patterns paralleling those found in human breast cancers (Herschkowitz, et al. 2007). In contrast, in the presence of *Esr1* over-expression 100% of *Tet-op-Esr1*$^{MMTV\text{-}rtTA}$/Brca1 floxed exon 11 $^{(f11)/f11/MMTV\text{-}Cre/p53+/-}$ mice develop mammary adenocarcinomas and 50% of these are ER+ (Table 1A). HAN prevalence is also 100% by 12 months of age and 50% of the hyperplasias are ER+ (Jones, et al. 2008). The mammary cancers that develop in the *tet-op-Esr1*$^{MMTV\text{-}rtTA/Brca1\ f11/f11/MMTV\text{-}Cre/p53+/-}$ mice most commonly histologically model human invasive ductal carcinoma.

Models that develop ERα+ cancer through alterations in molecules interacting with the estrogen-signaling pathway

Cyclin D1 over-expression mouse models

Cyclin D1 plays an important role in regulating estrogen signaling in mammary tissue (Casimiro, et al. 2013; Fu, et al. 2004). Expression of Cyclin D1 is positively correlated with ERα expression in breast cancer (Bostrom, et al. 2009; van Diest, et al. 1997). Cyclin D1 forms a complex with CDK4 or CDK6 to regulate the cell cycle at the G1/S phase and CDK 4/6 inhibitors such as PD 0332991 are being studied in combination with anti-hormonal agents for treatment of ERα+ cancer (Lange and Yee 2011; Sutherland and Musgrove 2002). Depending upon the length of time observed, specific transgenic line and strain background studied, cyclin D1 over-expression targeted to mammary epithelial cells in *MMTV-Ccdn1* transgenic mice results in development of mammary cancer in 5% to 47.5% of mice (Lin, et al. 2008; Miermont et al. 2012; Wang, et al. 1994) (Table 1B). Adenocarcinoma and adenosquamouscarcinoma histologic types appear, corresponding to the same histologic sub-types in invasive human breast cancers. On an FVB background, 47.5% of *MMTV-Ccdn1* mice develop mammary cancer between 20 and 23 months of age, 37.5% of these cancers are ER+, and isolated ER+ cancer cells placed in tissue culture are reported to demonstrate estrogen responsiveness (Lin, et al. 2008). *MMTV-Ccdn1*^{T286A} transgenic mice carry a genetically engineered cyclin D1 allele with an activating mutation. The mutant cyclin D1 encoded by the genetically engineered allele cannot be phosphorylated. This lack of phosphorylation interrupts cyclin D1 nuclear export resulting in its retention in the nucleus and continuing activity (Lin et al. 2008). On an FVB background, 51% of these mice develop mammary cancer by 16–20 months of age, 50% are ER+, and the isolated cancer cells in tissue culture demonstrate estrogen responsiveness and tamoxifen growth inhibition (Lin et al. 2008). A high proportion of mammary adenocarcinomas arising in the *MMTV-Ccdn1* mice demonstrate papillary histology modeling human papillary breast cancer while secretory glandular histology predominates in *MMTV-Ccdn1*^{T286A} mice, an uncommon histological subtype in humans.

Prolactin over-expression mouse models

Prolactin is a peptide hormone essential for normal mammary growth and development that, when over-expressed in mammary tissue, can induce mammary cancer development in mouse models. In women the role of prolactin in breast cancer development remains under investigation (Clevenger, et al. 2003; Tworoger, et al. 2013). Prolactin has the ability to activate ERα in the absence of ligand (O'Leary, et al. 2013) and high levels of prolactin have been associated with tamoxifen and aromatase inhibitor resistance (Barni, et al. 1998; Bhatavdekar, et al. 1994; Dowsett, et al. 1983). Genetically engineered mice over-expressing rat prolactin (Prl) from the hormonally unresponsive rat neu-related lipocalin (Nrl) promoter in mammary epithelial cells were generated to study the potential role of local prolactin over-expression in mammary cancer (Arendt, et al. 2011; Rose-Hellekant, et al. 2003) (Table 1B). ER+ adenocarcinomas developed in one of the two FVB/N Prl-Nrl founder lines generated (Line 1655–8) (Table 1B). Line 1655–8 demonstrates elevated levels of serum prolactin and 80% of the female mice from this line develop mammary adenocarcinomas between 12 and 21 months of age and 50% of the cancers are ER+. Papillary is the predominant histology (44%) followed by glandular (22%) and solid (22%). Different percentages of ER+ cancer cells are described in the different cancer histologies: glandular (10%), solid (4%), papillary (21%), adenosquamous (32%), and carcinosarcoma (8%), modeling different histologic sub-types of some of the less commonly diagnosed human ER+ breast cancers. Significantly, aged (~22 months of age) FVB/N mice with pituitary prolactinomas also develop ER+ mammary cancers (Radaelli, et al. 2009).

Wnt-1 mouse models

Molecules involved in the Wnt-1 signaling pathway (including cyclin D1, c-myc, and β-catenin) have been implicated in breast cancer (Li, et al. 2000). Wnt-1 signaling increases β-catenin levels, which transcriptionally activates cyclin D1 and c-myc. The impact of Wnt-1 signaling on cyclin D1 ultimately affects the downstream estrogen-ER complex that regulates gene transcription and expression. The role of Wnt1 in mammary cancer was initially investigated because it is one of the randomly selected sites of integration for the mouse mammary tumor virus. Eighty percent of female *MMTV-Wnt1* transgenic mice develop mammary cancer between three and seven months of age with ~86% categorized as ER+ as defined by at least 5% of the mammary epithelial cancer cells demonstrating ERα expression (Zhang, et al. 2005) (Table 1B). Growth of the cancers, however, is not repressed by loss of estrogen signaling. Instead ER+ cells are lost and selection of proliferating ER negative cells maintains cancer growth. Histology of the ER+ mammary cancers was not defined. While neither Ras mutation or PTEN insufficiency impact the percentage of Wnt1-induced mammary cancers demonstrating ER expression, *p53* haploinsufficincy, *p53* insufficiency and HER2/Neu over-expression lead to loss of ER positivity (Fuchs-Young, et al. 2011; Zhang et al. 2005).

p53 mutation, p53 deletion and p53 deletion transplant mouse models

Mutations in the p53 gene are reported in 20–23% of ER positive breast cancer and are reported to negatively impact response to anti-hormonal therapy (Gasco, et al. 2002; Uji, et al. 2013; Yamamoto, et al. 2013). A variety of cellular processes regulated by p53, including

cell cycle control, apoptosis, senescence and response to DNA damage, can affect breast cancer development and therapy response (Lai, et al. 2012; Walerych, et al. 2012). *In vitro* p53 has been shown to regulate ERα transcription by recruiting essential transcription factors to the ERα promoter in MCF-7 cells (Shirley, et al. 2009). However, *In vivo*, *p53* deficient mice have been shown to develop ER+ tumors indicating that p53 is not required for ER expression.

Three different mouse models of functional *p53* loss develop ER+ mammary cancer (Table 1B). Expression of a p53 R270H mutant allele targeted to mammary epithelium and activated during pregnancy using a *WAP-Cre* transgene (Wijnhoven, et al. 2005) generates a mouse model in which mammary cancers appeared in 87% of the mice with a mean latency of five months following activation of Cre recombination during pregnancy. Sixty-seven percent of the cancers are reported to exhibit ER stained cells. Papillary and carcinosarcoma subtypes, modeling two less commonly seen human histologic sub-types, demonstrate epithelial cell staining and a sarcoma subtype shows only positive mesenchymal cells. In another model, the *WAP-cre* transgene is used to delete both copies of *p53* in mammary epithelium (Lin et al. 2004). Ninety-two percent of the parous mice develop mammary tumors with a median tumor latency of 9.5 months. Forty percent of the cancers are ER+ and include adenocarcinoma, myoepithelial adenocarcinoma, adenosquamous carcinoma, and spindle cell histologies. The adenocarcinomas would model human ductal carcinoma while the others would model less frequently diagnosed sub-types. In contrast, if an *MMTV-Cre* transgene is used to execute the *p53* deletion, none of the mammary cancers that develop are ER+, whether they are parous or virgin. A third p53-related model developed in BALB/c mice uses implants of mammary epithelium from eight-week-old female mice with germ-line loss of *p53* that are placed into the cleared mammary fat pads of three week old mice to generate a mouse model of human DCIS (Jerry, et al. 2000; Medina, et al. 2003; Medina, et al. 2002). Between 24% and 55% of the implanted mice develop disease by 11 or 12 months following implantation and 21% of the lesions are ER+.

Signal transducers and activators of transcription factor (STAT) 1 deficient mouse model

STAT 1 plays a role in mediating innate immunity, lying downstream of type I and II interferons, and is reported to both promote leukemogenesis and inhibit mammary carcinogenesis in mice (Koromilas and Sexl 2013). STAT 1 is expressed in human breast cancer epithelial cells with some, but not all, studies demonstrating a positive correlation with ERα and disease prognosis (Furth 2013). Ninety percent of the mammary carcinomas that develop in STAT1-deficient (129S6/SvEvTac-*Stat1*^{tm1Rds}) mice are ER+, show hormone dependent growth, and demonstrate luminal-type cancer surface markers (Chan, et al. 2012) (Table 1B). Mammary cancers develop between 18 and 26 months of age. While only 62% of nulliparous mice develop mammary cancer, this increases to 91% in multiparous mice. The molecular signature of the mammary carcinomas developing in the mice resembles that of human luminal-type breast cancers. Histology is only specified as carcinoma and not further sub-typed. Follow-up studies suggest that Stat1 suppresses mammary cancer formation through regulation of Janus kinase (JAK)2 activity by suppressor of cytokine signaling (SOCS)1 (Chan, et al. 2013).

Transforming growth factor alpha (TGFα) over-expression mouse model

TGFα is a member of the epidermal growth factor family that is overexpressed in some human breast cancers (Booth and Smith 2007). It promotes epithelial development and proliferation. Upon binding to its receptor, ErbB1, it creates either a homo-dimer or heterodimer with another member of the ErbB family of proteins (Roepstorff, et al. 2008). Some of the dimers have been observed in ER− or ER+/PR− tumors spurring an interest in the role of TGFα in breast cancers (Holbro, et al. 2003). TGFα expression can be found in 50–70% of human breast tumors and has been found to be downregulated by tamoxifen in ER+/PR+ breast cancers (Ciardiello, et al. 1991; Noguchi, et al. 1993). TGFα signaling stimulates cytoplasmic PI3K, which triggers Akt. Genetically engineered FVB/N mice that over-express TGFα in mammary epithelial cells due to an *Nrl-TGFα* transgene will develop ER+ mammary cancers (Rose-Hellekant, et al. 2007) (Table 1B). It is reported that “most” mammary cancers are ER+ but PGR-, appear in virgin mice between ages 9 and 21 months and parous mice between ages 8–14 months of age. Cystic papillary histopathology is found in all the mice with development of “solid adenomatous” tumors in some mice, the first type may correspond to the papillary sub-type in human. Ovariectomy reduces mammary tumor incidence from 100% in mice with ovaries to 67% in ovariectomized mice and increased the mean age at which tumors appeared by four months. Significantly mammary cancers that appear in *Nrl-TGFα/p53+/−* mice are ER negative.

AIB1 over-expression mouse model

MMTV-AIB1 mice on an FVB/N background were generated to test the impact of high levels of AIB1 expression targeted to mammary epithelial cells (Torres-Arzayus, et al. 2004) (Table 1B). ER+ and ER− mammary tumors appear in 40% and 8%, respectively, of female mice with the majority appearing between 12 and 25 months of age. There was no significant difference in time of onset or incidence between virgin and parous mice. Both mammary preneoplasia and tumors are reported to demonstrate high levels of the phosphorylated forms of Insulin Growth Factor-I Receptor, the p70S6 kinase, and phospho-S6 ribosomal protein. Other tumors that appear in these mice include the frequent appearance of pituitary adenomas, uterine leiomyosarcomas, and lung adenocarcinomas and less frequently fibrosarcomas, skin papillomas and squamous cell carcinomas, ovarian teratomas, lymphomas, osteomacrophage sarcomas, and adrenocortical tumors of the kidney. ER+ tumor histopathology is reported as microacinar and comedo type, types corresponding to non-invasive DCIS-type breast cancers in women.

Separase over-expression mouse model

Espl1 encodes the gene for separase, a cysteine protease that hydrolyzes cohesin, mediating progression from metaphase to anaphase. It is over-expressed in some ER+ human breast cancers. Eighty percent of multiparous transgenic C57Bl/6 mice carrying an *MMTV-Espl1* transgene develop mammary tumors by 10–11 months of age (Mukherjee, et al. 2013) (Table 1B). Tumors also develop in primiparous but not nulliparous mice. Nuclear and cytoplasmic staining for ER is described in all histological types of mammary cancers that developed in these mice: spindle-like, squamous, solid, and glandular, the first two sub-

types representing less common histologic human breast cancer sub-types with further information required to know if the other histologies correspond to human ductal carcinomas. Significant intra- and inter-tumor heterogeneity demonstrating both luminal and basal features is present with more and less differentiated areas exhibiting different ER expression levels. An immune reaction with hyperproliferative stroma is present in 12-month-old mice. Introduction of a *p53/−* background into the mice did not significantly alter tumor penetrance or latency, however lung metastases are found only in *MMTV-Espl1/p53+/−* mice and the percentage of ER+ cells is reduced to ~45%. The *MMTV-Espl1/p53+/−* model is described as being representative of the more aggressive forms of human breast cancer that exhibit genomic instability, cell cycle defects and metastases.

Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (*Pik3ca*) gain of function mutation mice

Activating mutations in the *PIK3CA* gene occur in between one quarter and one third of human breast cancers with 40% of these mutations located to the kinase domain. Introduction of a *Pik3ca* gene sequences carrying the *H1047R* activating mutation (*Pik3ca*^{H1047R}) into the *ROSA26* locus with activation through expression of an *MMTV-Cre* transgene results in 43% of the *Pik3ca*$^{H1047R/p53f/+/MMTV\text{-}CreLineA}$ mice developing mammary tumors by 10 months of age when the more strongly expressed *MMTV-Cre*$^{Line\ A}$ transgene is used, and 69% of mice by 17 months of age when the more mammary cell targeted but heterogeneously expressed *MMTV-Cre*^{NLST} transgene is used (Table 1B) (Adams, et al. 2011). Lymphomas/thymomas as well as other tumors also develop in these mice at significant frequencies (84% and 43%, respectively). Tumors appear in both virgin and parous mice. Loss of one *p53* allele increases the percentage of *Pik3ca*$^{H1047R/p53f/+/MMTV\text{-}CreNLST}$ mice demonstrating mammary tumors to 80% by approximately 10 months of age. Ninety-six percent of the tumors developing in the *Pik3ca*$^{H1047R/p53f/+/MMTV\text{-}CreNLST}$ mice are reported as either adenomyoepithelioma or adenosquamouscarcinoma and ER+ cells are noted to be found in each of these histological cancer types, although specific percentages are not reported. These represent some of the less commonly seen ER+ histological sub-types found in women. Spindle cell tumors also appear.

Pharmacological interventions can promote development of ERα+ mammary cancer in genetically engineered mice

PPARγ is expressed in human breast cancers where, when activated, it can exert a differentiating, apoptotic and/or growth inhibiting effect (Kotta-Loizou, et al. 2012). Efatutazone was tested as a cancer preventative in BRCA mice because it is a high affinity PPARγ agonist that does not demonstrate activation of related receptors PPARα or PPARΔ and is currently in human clinical trials for cancer therapy. BRCA mice were used because they have intrinsic resistance to tamoxifen (Jones et al. 2008) and efatutazone represents an alternative preventative. The percentage of *Brca1*$^{f11\ (f11)/f11/MMTV\text{-}Cre/p53+/-}$ mice developing ERα+ cancers can be increased by exposure to the peroxisome proliferator-activated receptor gamma agonist efatutazone (Nakles, et al. 2013) (Table 1C). While

expression of ERα is rare (0–1%) in cancers developing in untreated Brca1$^{f11/f11/MMTV\text{-}Cre/p53+/-}$ mice, it rises to 23% in cancers that develop on efatutazone treatment initiated at 4 months of age. ER+ histologic sub-types that appear on efatutazone in Brca1$^{f11/f11/MMTV\text{-}Cre/p53+/-}$ mice include papillary and DCIS, modeling the same histological sub-types found in women.

Carcinogen exposure can promote development of ERα+ mammary cancer in genetically engineered mice

Nuclear localization of phosphorylated V-Akt Murine Thymoma Viral Oncogene Homolog 1 (AKT1) is correlated with ER positivity in human breast cancers (Bostner, et al. 2013). AKT1 lies downstream of Phosphoinositide-3-Kinase (PI3K) signaling. This pathway can play numerous roles in carcinogenesis (Klarenbeek, et al. 2013). To explore the role of AKT1 activation in breast cancer, a genetically engineered mouse model with expression of an artificially constitutively activated form of AKT1 targeted to mammary epithelium using the MMTV promoter was generated (*MMTV-myrAkt1* mice) (Blanco-Aparicio, et al. 2007) (Table 1D). Localization of AKT1 to the membrane through myristoylation generates a mouse model with constitutive AKT activation. Both lines of transgenic mice generated develop mammary cancer, but only after exposure to the chemical carcinogen, 7,12-dimethylbenz(a)anthracene (DMBA) beginning at nine weeks of age and continuing for five weeks. Roughly 40% of the mice exhibit either mammary adenocarcinoma (papillary or poorly differentiated) or adenosquamous carcinoma between 13 and 39 weeks after DMBA administration (between six and 12 months of age). All of the cancers are ER+. The poorly differentiated cancers may model the more commonly found invasive ductal carcinomas in women while the other two sub-types would model less commonly found morphologies. Given the cytoplasmic localization of Akt1 in these transgenic mice, they do not directly model the nuclear Akt1 localization reported in human ER+ breast cancer. However, wild-type mice exposed to DMBA demonstrate predominantly ER negative mammary tumors (Yin, et al. 2009). The appearance of ER+ mammary cancers in this model appears to be functionally related to expression of the activated Akt1.

Spontaneous ER+ mammary cancer nude mouse model

Although not deliberately genetically engineered, brother-sister matings of heterozygous NIH nude mice resulted in development of a line of mice with high serum estrogen levels in which 62% of females develop ERα positive metastatic mammary cancers by a mean age of seven months (Kumar, et al. 2007) (Table 1E). Mammary adenocarcinomas appear only in breeding females. Loss of ovarian hormone stimulation through ovariectomy leads to tumor regression. Histologically the tumors are described as having tubular features, one of the less common ER+ sub-types found in women, generally associated with a good prognosis.

Summary and Conclusions

Here we describe a spectrum of genetically engineered mouse models that develop ER+ mammary cancer. Different strain backgrounds are represented. While there is significant variability in the percentage of cancer cells demonstrating ER positivity, reported levels fall

within the criteria used to define ER+ breast cancer. Some, but not all, of the models have been tested to determine their response to anti-hormonal agents and/or investigated for hormone dependency for growth. An important step in validating genetically engineered mouse models of different breast cancer subtypes is to compare their transcriptional profiles with those found in human breast cancers (Pfefferle, et al. 2013). ER+ breast cancer models that have been more rigorously investigated for parallels to human disease include Wnt-1 over-expression and *BRCA1, STAT1* and *p53* deficient models. The long latency (>12 months of age) of many models renders them challenging and expensive to work with, however application of mammary epithelial transplant techniques could make them more tractable for study. There does not appear to be one best mouse model of ER+ mammary cancer consistent with the fact that there is not one type of ER+ human breast cancer. Instead, like all experimental tools, the model system selected should be that most suitable for the experimental design and goals. For example, if there were reason to directly control the timing of ERα expression or co-express ERα with another gene, then a conditional system would be most appropriate. If the goal is to determine factors that regulate appearance or maintenance of ERα expression in mammary cancers, then one of the spontaneous ERα+ models may be more useful. More uniform and comprehensive information on hormone responsiveness, response to anti-hormonal agents, and gene expression patterns as compared to human ER+ breast cancers as well as characterization of the genetically engineered mice on different strain backgrounds would be useful.

Future Directions

Opportunity for generation of new genetically engineered mouse models remains. There is a strong need for ER+ models that reliably develop metastatic disease. Further characterization of the model developed in nude mice (Kumar et al. 2007) is required before it can be effectively used for experiments that might address the pathophysiology of ER+ metastatic disease. Another approach to developing more sophisticated models reflective of individual ER+ breast cancer sub-types will be to combine transgenic *Esr1* expression with other genetic manipulations as was accomplished for both TAg expression and loss of *Brca1/p53*. For example combining *Tet-op-Esr1*$^{MMTV\text{-}rtTA}$ mice with a mouse model of ErbB2/HER2 mutation (Ursini-Siegel, et al. 2007) could generate a model for Luminal B ER + breast cancer. Although joining Esr1 over-expression with germ-line *p53* haploinsufficiency did not accelerate tumor development (Diaz-Cruz and Furth 2010), combining *p53*$^{R270H/+}$/WAP-Cre with *Tet-op-Esr1*$^{MMTV\text{-}rtTA}$ mice might be more potent as mutant *p53* ^{R270H} has more molecular impact than simple reduction of *p53* expression levels. Moreover, a model with mutant *p53* would be translationally relevant for breast cancers carrying somatic p53 mutation in contrast to the germ-line insufficiency model that more closely parallels Li–Fraumeni syndrome. Bringing Esr1 over-expression into the NRL-PRL Line 1655–8 model could make a new laboratory tool for further study of the epidemiologically defined risk of elevated prolactin on breast cancer (Tworoger et al. 2013). Loss of *Stat1* accelerates mammary cancer development in *MMTV-Neu-IRES-Cre* mice (Klover, et al. 2010). If loss of *Stat1* in *Tet-op-Esr1*$^{MMTV\text{-}rtTA}$ mice accelerated cancer development to under 12 months, this would be a more tractable model for further study of luminal type breast cancer. Targeted development of new models and refinement of existing

models will need to build upon the new information emerging from deep sequencing and genetic characterization of ER+ breast cancer in humans.

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Table 1

Genetically engineered mouse models that develop ER+ breast cancer

A. Models developing ER+ mammary cancer from direct over-expression of ERα							
Published Nomenclature	**Genetic Nomenclature**	**Background Strain**	**Age range of mice demonstrating cancer development (months)**	**Percentage of mice with mammary cancers within this age range**	**Percentage of mammary cancers designated ER+**	**Parity required for tumor development**	**Refs**
tTA/TAg/ER-α	*Tet-op-Esr1*$^{MMTV\text{-}tTA/tet\text{-}op\text{-}SV40\text{-}TAg}$	C57Bl/6	10–12	37%	100%	No	Tilli MT, Frech MS, Steed ME, Hruska KS, Johnson MD, Flaws JA & Furth PA 2003 Introduction of estrogen receptor-alpha into the tTA/TAg conditional mouse model precipitates the development of estrogen-responsive mammary adenocarcinoma. *Am J Pathol* **163** 1713–1719.
CERM (Conditional Estrogen Receptor alpha in Mammary tissue)	*Tet-op-Esr1*$^{MMTV\text{-}rtTA}$	C57Bl/6	10–12	3–5%	50%	No	Miermont AM 2012 Association of Over-Expressed Estrogen Receptor Alpha with Development of Tamoxifen Resistant Hyperplasia and Adenocarcinomas in Genetically Engineered Mice. *Anatomy & Physiology* **02**. Miermont AM, Parrish AR & Furth PA 2010 Role of ERalpha in the differential response of Stat5a loss in susceptibility to mammary preneoplasia and DMBA-induced carcinogenesis. *Carcinogenesis* **31** 1124–1131. Frech MS, Halama ED, Tilli MT, Singh B, Gunther EJ, Chodosh LA, Flaws JA & Furth PA 2005 Deregulated

A. Models developing ER+ mammary cancer from direct over-expression of ERα							
Published Nomenclature	**Genetic Nomenclature**	**Background Strain**	**Age range of mice demonstrating cancer development (months)**	**Percentage of mice with mammary cancers within this age range**	**Percentage of mammary cancers designated ER+**	**Parity required for tumor development**	**Refs**
							estrogen receptor alpha expression in mammary estrogen receptor alpha expression in mammary estrogen receptor alpha expression in mammary estrogen receptor alpha expression in mammary estrogen receptor alpha expression in mammary estrogen receptor alpha expression in mammary *Clin Cancer Res* 65 681–685.
AIB1Δ3/CERM	*Tet-op-Esr1*$^{MMTV\text{-}tTA/tet\text{-}op\text{-}AIB1\Delta3}$	C57Bl/6	19–26	7%	50%	No	Nakles RE, Shiffert MT, Diaz-Cruz ES, Cabrera MC, Alotaiby M, Miermont AM, Riegel AT & Furth PA 2011 Altered AIB1 or AIB1Delta3 expression impacts ERalpha effects on mammary gland stromal and epithelial content. *Mol Endocrinol* **25** 549–563.
Brca1$^{f/f;MMTV\text{-}Cre/p53+/-/CERM}$	*Tet-op-Esr1*$^{MMTV\text{-}rtTA/Brca1\ f11/f11/MMTV\text{-}Cre/p53+/-}$	C57Bl/6	9–16	100%	50%	No	Jones LP, Assefnia S, Chandrasekharan S, et al. 2007 Identification of conserved gene expression features between murine mammary carcinoma models and human breast tumors. *Genome Biol* **8** R76.

B. Models developing ER+ mammary cancer as a result of genetic alterations of molecules impacting estrogen signaling							
Published Nomenclature	**Genetic Nomenclature**	**Background Strain**	**Age range of mice demonstrating cancer development (months)**	**Percentage of mice with mammary cancers within this age range**	**Percentage of mammary cancers designated ER+**	**Parity required for tumor development**	**Refs**
MMTV-cyclinD1	*MMTV-Ccdn1*	C57Bl/6	12	5%	100%	No	Miermont AM 2012 Association of Over-Expressed Estrogen Receptor Alpha with Development of Tamoxifen Resistant Hyperplasia and

B. Models developing ER+ mammary cancer as a result of genetic alterations of molecules impacting estrogen signaling							
Published Nomenclature	**Genetic Nomenclature**	**Background Strain**	**Age range of mice demonstrating cancer development (months)**	**Percentage of mice with mammary cancers within this age range**	**Percentage of mammary cancers designated ER+**	**Parity required for tumor development**	**Refs**
							Adenocarcinomas in Genetically Engineered Mice. Adenocarcinomas in Genetically Engineered Mice. *Anatomy & Physiology* **02**
							Wang TC, Cardiff RD, Zukerberg L, Lees E, Arnold A & Schmidt EV 1994 Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice. *Nature* **369** 669–671.
MMTV-cyclinD1	*MMTV-Ccdn1*	FVB	20–23	47.5%	37.5%	Yes	Lin DI, Lessie MD, Gladden AB, Bassing CH, Wagner KU & Diehl JA 2008 Disruption of cyclin D1 nuclear export and proteolysis accelerates mammary carcinogenesis. *Oncogene* **27** 1231–1242.
MMTV-D1T286A	*MMTV-Ccdn1*^{T286A}	FVB	16–20	51%	50%	Yes	Lin DI, Lessie MD, Gladden AB, Bassing CH, Wagner KU & Diehl JA 2008 Disruption of cyclin D1 nuclear export and proteolysis accelerates mammary carcinogenesis. *Oncogene* **27** 1231–1242.
NRL-PRL Line 1655-8	*Lcn2-Prl*$^{1655–8}$	FVB	12–21	80%	50%	No	Rose-Hellekant TA, Arendt LM, Schroeder MD, Gilchrist K, Sandgren EP & Schuler LA 2003 Prolactin induces ERalpha-positive and ERalpha-negative mammary cancer in transgenic mice. *Oncogene* **22** 4664–4674.
MMTV-Wnt1	*MMTV-Wnt1*	FVB	3–7	80%	86%	No	Zhang X, Podsypanina K, Huang S, Mohsin SK, Chamness GC, Hatsell S, Cowin P, Schiff R & Li Y 2005 Estrogen receptor positivity in mammary tumors of Wnt-1 transgenic mice is influenced by collaborating oncogenic mutations. *Oncogene* **24** 4220–4231.
P53(R270H/+)WAPCre	*p53*$^{R270H/+/WAP\text{-}Cre}$	129Sv/C57BL/6	7–8	87%	67%	Yes	Wijnhoven SW, Zwart E, Speksnijder EN, Beems RB, Olive KP, Tuveson DA, Jonkers J, Schaap MM, van den Berg J, Jacks T, et al. 2005 Mice expressing a mammary gland-specific R270H mutation in the p53 tumor suppressor gene mimic human breast cancer development. *Cancer Res* **65** 8166–8173.

B. Models developing ER+ mammary cancer as a result of genetic alterations of molecules impacting estrogen signaling							
Published Nomenclature	**Genetic Nomenclature**	**Backgroun d Strain**	**Age range of mice demonstrating cancer development (months)**	**Percentage of mice with mammary cancers within this age range**	**Percentage of mammary cancers designated ER+**	**Parity required for tumor development**	**Refs**
P53$^{fp/fp}$WAPCre^{c}	*p53$^{fp/fpWAPCrec}$*	CB6F1 × C57BL/6	8–12.5	92%	40%	No	Lin SC, Lee KF, Nikitin AY, Hilsenbeck SG, Cardiff RD, Li A, Kang KW, Frank SA, Lee WH & Lee EY 2004 Somatic mutation of p53 leads to estrogen receptor alpha-positive and -negative mouse mammary tumors with high frequency of metastasis. *Cancer Res* **64** 3525–3532.
p53 null	*p53*	BALB/c	11–12	24–55%	21%	No	Medina D, Kittrell FS, Shepard A, Stephens LC, Jiang C, Lu J, Allred DC, McCarthy M & Ullrich RL 2002 Biological and genetic properties of the p53 null preneoplastic mammary epithelium. *Faseb j* **16** 881–883.
Stat1$^{-/-}$	129S6/SvEvTac-*Stat1^{tm1Rds}*	129S6/SvEv	18–26	62%	90%	No	Chan SR, Vermi W, Luo J, Lucini L, Rickert C, Fowler AM, Lonardi S, Arthur C, Young LJ, Levy DE, et al. 2012 STAT1-deficient mice spontaneously develop estrogen receptor alpha-positive luminal mammary carcinomas. *Breast Cancer Res* **14** R16.
TGFα×*p53*+/−	*Nrl-TGFα$^{p53+/-}$*	FVB/N	9–21	100%	“most”	No	Rose-Hellekant TA, Schroeder MD, Brockman JL, Zhdankin O, Bolstad R, Chen KS, Gould MN, Schuler LA & Sandgren EP 2007 Estrogen receptor-positive mammary tumorigenesis in TGFalpha transgenic mice progresses with progesterone receptor loss. *Oncogene* **26** 5238–5246.
MMTV-AIB1	*MMTV-AIB1*	FVB/N	12–25	76%	40%	No	Torres-Arzayus MI, Font de Mora J, Yuan J, Vazquez F, Bronson R, Rue M, Sellers WR & Brown M 2004 High tumor incidence and activation of the PI3K/AKT pathway in transgenic mice define AIB1 as an oncogene. *Cancer Cell* **6** 263–274.
MMTV-Espl1	*MMTV-Espl1*	C57Bl/6	10–11	80%	100%	Yes	Mukherjee M, Ge G, Zhang N, Edwards DG, Sumazin P, Sharan SK, Rao PH, Medina D & Pati D 2013 MMTV-Espl1 transgenic mice develop aneuploid, estrogen receptor

B. Models developing ER+ mammary cancer as a result of genetic alterations of molecules impacting estrogen signaling							
Published Nomenclature	**Genetic Nomenclature**	**Background Strain**	**Age range of mice demonstrating cancer development (months)**	**Percentage of mice with mammary cancers within this age range**	**Percentage of mammary cancers designated ER+**	**Parity required for tumor development**	**Refs**
							alpha (ERalpha)-positive mammary adenocarcinomas. alpha (ERalpha)-positive mammary adenocarcinomas. *Oncogene*
MMTV-Espl1, p53+/−	*MMTV-Espl1*$^{p53+/-}$	C57Bl/6	10–11	100%	45%	Yes	Mukherjee M, Ge G, Zhang N, Edwards DG, Sumazin P, Sharan SK, Rao PH, Medina D & Pati D 2013 MMTV-Espl1 transgenic mice develop aneuploid, estrogen receptor alpha (ERalpha)-positive mammary adenocarcinomas. *Oncogene*
Pik3ca^{H1047R}	R26-Pik3ca$^{H1047R/MMTV-CreNLST}$	FVB	5–16	69%	96%	Yes	Adams JR, Xu K, Liu JC, Agamez NM, Loch AJ, Wong RG, Wang W, Wright KL, Lane TF, Zacksenhaus E, et al. 2011 Cooperation between Pik3ca and p53 mutations in mouse mammary tumor formation. *Cancer Res* **71** 2706–2717.

C. Model developing ER+ mammary cancer as a result of pharmacologic treatment in combination with genetic alterations of molecules impacting estrogen signaling								
GEM Model Published Nomenclature	**Genetic Nomenclature**	**Background Strain**	**Age range of mice demonstrating cancer development (months)**	**Percentage of mice with mammary cancers within this age range**	**Percentage of mammary cancers designated ER+**	**Pharmacological Inducer**	**Parity required for tumor development**	**Ref**
Brca1$^{f11/f11/p53+/-/MMTV-Cre}$	*Brca1*$^{f11/f11/p53+/-MMTV-Cre}$	C57Bl/6	10–12	100%	23%	Efatutazone administered at age 4 months	No	Nakles RE, Kallakury BV & Furth PA 2013 The PPARgamma agonist efatutazone increases the spectrum of well-differentiated mammary cancer subtypes initiated by loss of full-length BRCA1 in association with TP53 haploinsufficiency. *Am J Pathol* **182** 1976–1985.

D. Model developing ER+ mammary cancer as a result of carcinogen exposure in combination with genetic alterations of molecules impacting estrogen signaling								
Published Nomenclature	**Genetic Nomenclature**	**Background Strain**	**Age range of mice demonstrating cancer development (months)**	**Percentage of mice with mammary cancers within this age range**	**Percentage of mammary cancers designated ER+**	**Chemical Inducer**		**Ref**
MMTV-myrAkt1	*MMTV-AKT1^{myr}*	C57Bl/6	6–12	40%	100	7,12-dimethylbenz(a)anthracene (DMBA) treatment for 5 weeks beginning at 9 weeks of age	No	Blanco-Aparicio C, Perez-Gallego L, Pequeno B, Leal JF, Renner O & Carnero A 2007 Mice expressing myrAKT1 in the mammary gland develop carcinogen-induced ER-positive mammary tumors that mimic human breast cancer. *Carcinogenesis* **28** 584–594

E. Model developing ER+ mammary cancer as a result of brother-sister matings of nude mice						
Published Nomenclature	**Background Strain**	**Age range of mice demonstrating cancer development (months)**	**Percentage of mice with mammary cancers within this age range**	**Percentage of mammary cancers designated ER+**	**Parity required for tumor development**	**Ref**
Spontaneous Mammary Tumor model	NIH Nude	3.5–12	62%	100%	Yes	Kumar MJ, Ponvijay KS, Nandhini R, Nagarajan RS, Jose J, Srinivas G, Nagarajan P, Venkatesan R, Kumar K & Singh S 2007 A mouse model for luminal epithelial like ER positive subtype of human breast cancer. *BMC Cancer* **7** 180.