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REVIEW

Current views on *in vivo* models for breast cancer research and related drug development

Tiago Ferreira [a,b*], Tiago Azevedo [a,b*], Jessica Silva [a,b*], Ana I. Faustino-Rocha [a,b,c,d] and Paula A. Oliveira [a,b,e]

[a]Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [b]Institute for Innovation, Capacity Building and Sustainability of Agri-Food Production (Inov4Agro), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal; [c]Department of Zootechnics, School of Sciences and Technology, University of Évora, Évora, Portugal; [d]Department of Zootechnics, School of Sciences and Technology, Comprehensive Health Research Center, Évora, Portugal; [e]Clinical Academic Center of Trás-Os-Montes and Alto Douro, University of Trás-Os-Montes and Alto Douro, Vila Real, Portugal

ABSTRACT

Introduction: Animal models play a crucial role in breast cancer research, in particular mice and rats, who develop mammary tumors that closely resemble their human counterparts. These models allow the study of mechanisms behind breast carcinogenesis, as well as the efficacy and safety of new, and potentially more effective and advantageous therapeutic approaches. Understanding the advantages and disadvantages of each model is crucial to select the most appropriate one for the research purpose.

Area covered: This review provides a concise overview of the animal models available for breast cancer research, discussing the advantages and disadvantages of each one for searching new and more effective approaches to treatments for this type of cancer.

Expert opinion: Rodent models provide valuable information on the genetic alterations of the disease, the tumor microenvironment, and allow the evaluation of the efficacy of chemotherapeutic agents. However, *in vivo* models have limitations, and one of them is the fact that they do not fully mimic human diseases. Choosing the most suitable model for the study purpose is crucial for the development of new therapeutic agents that provide better care for breast cancer patients.

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1. Breast cancer

Breast cancer is one of the most commonly occurring cancers worldwide, affecting about 10% of women during their lifetime [1]. Although it can affect anyone, regardless of age, sex, race or ethnicity, some groups experience higher incidence and mortality rates than others, especially African American and Hispanic female population [2]. The main risk factors for breast cancer include older age and being a woman [3], a family history of breast cancer, genetic mutations in high penetrance genes, hormone exposure, lifestyle (alcohol consumption, obesity, sedentarism, not breastfeeding, menopause), and reproductive history (early menarche and nulliparous women) [4,5].

Breast cancer is a highly heterogeneous disease [6], occurring more commonly in the terminal duct-lobular unit [7], and exhibits both intra- and inter-tumor heterogeneity [8]. Breast tumors can be classified into subtypes based on characteristics such as histopathology [9], molecular subtype [10], tumor grade [11], and tumor, node, and distant metastasis (TNM) stage [12]. More than 40 different histological subtypes are recognized by the World Health Organization for the classification of breast tumors, based on cell morphology, growth, and architectural patterns, with the most common being the invasive ductal breast carcinoma of no special type [13,14].

Regarding molecular subtypes, breast cancer is classified according to the expression of specific genes, proteins, and cell receptors [15,16]. The key molecular subtyping focuses on Estrogen Receptor (ER), Progesterone Receptor (PR), and Human Epidermal Growth Factor Receptor 2 (HER2). The commonly recognized molecular subtypes of breast cancer based on the immunohistochemical expression of ER, PR and HER2 receptors status, include Luminal A (ER^+, PR^+, $HER2^-$), Luminal B (ER^+, PR^-, $HER2^{\pm}$), HER2 enriched (ER^-, PR^-, $HER2^+$) and Triple-negative (ER^-, PR^-, $HER2^-$) [17].

The understanding of the molecular pathways behind the onset and progression of breast cancer has been constantly evolving due to continued research [18]. Experimental models for studying breast cancer and assessing prospective treatments are generally conducted *in vitro* and *in vivo* [15]. The use of cell lines offers a simpler and more practical method of analyzing the specific effect of a substance on various parameters, including cell viability, cell proliferation, colony formation, cytotoxicity, cytostasis, induction of apoptosis, and cell cycle arrest [19], and is in compliance with the 3 R's principle (reducement, refinement and replacement) that intend to reduce the use of *in vivo* models as much as possible. However, *in vitro* models are not able to preserve original

CONTACT Paula A. Oliveira pamo@utad.pt Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), Vila Real 5000-801, Portugal
*These authors contributed equally.

Article highlights

- Animal models, particularly mice and rats, are vital for breast cancer research as they closely resemble human mammary tumors, enabling the study of carcinogenesis mechanisms and the evaluation of new therapeutic approaches.
- Despite the similarities in tumor development and response to treatment, there are inherent biological and physiological differences between rodents and humans.
- Understanding the advantages and disadvantages of different animal models is crucial for selecting the most appropriate one for breast cancer research.
- The chemical carcinogen that is most used for inducing mammary cancer in animal models is 7,12-dimethylbenz[a]anthracene (DMBA).
- The use of patient-derived xenograft (PDX) models is a valuable approach for studying molecular subtypes of breast cancer.

cells' phenotype, cell-cell and cell-material interactions, which significantly contributes to an ineffective pre-clinical to clinical translation [20].

Animal models have been crucial to gain new knowledge about breast cancer [21]. The ongoing research into breast cancer aims to provide new and better therapies, improve early diagnosis, and ultimately find a cure for this condition. Animal models make possible to explore not only carcinogenesis mechanisms, but also to conduct preclinical research on new therapeutic approaches. The purpose of this paper is to review the animal models more frequently used to find new drugs for breast cancer treatment.

2. Animal models

The history and development of basic and translational breast cancer research in humans have been significantly influenced by animal models [22]. It was recognized, more than 2,400 years ago, that we could learn much about ourselves by studying animals. The concept of animal model was first defined in 1976 by Stanford Wessler as a 'living organism with an inherited, naturally acquired, or induced pathological process that in one or more respects closely resembles the same phenomenon occurring in Man' [23]. The use of animal models enables the study of physiological and pathological processes in a controlled environment [24], seeing as they share many biological similarities with humans [25].

Many non-mammalian species are utilized in breast cancer research to mimic the development, migration, and metastasis of breast cancer cell lines, including *Caenorhabditis elegans*, *Drosophila* spp. and *Danio rerio* (commonly known as zebrafish) [22]. Even though the quick reproductive cycles of these species make them useful for experimentation, they differ substantially from humans and lack many homologous genes, which constitute a huge limitation on their use [22]. Among mammalians, rodents, dogs, cats, pigs, treeshrews, and non-human primates are commonly used for breast cancer research [21]. However, owing to their small size, low cost of acquisition and maintenance, short generation time, and mature gene editing technologies, rodents, mice and rats are the most preferred species [25]. It is also worth to note that the use of mice and rats is less complex when compared to larger animals, like dogs, cats, and non-human primates, because there are less ethical, economic, and practical issues at stake [21].

Mice and rats share many similarities with humans in terms of anatomy, biochemistry, physiology, and genetics, and in this way, the mammary tumors developed by these animals exhibit similar characteristics with those of humans, including their morphology, histopathology and molecular signatures [26,27]. Various strains of mice and rats are available for research, including both inbred and outbred strains, each one with advantages and disadvantages. Inbred individuals are genetically identical with stable phenotypes, developing the same type of tumors at the same stage, while outbred animals have nonuniform genetic backgrounds, and develop different types of tumors at different ages [28]. It is worth to note that inbred strains provide a more controlled and reproducible research environment, leading to improved statistical power, while outbred strains better simulate the genetic diversity of human populations, potentially yielding interesting results [29]. Despite their genetical background, the models of mammary cancer may be categorized according to the way of induction, including: spontaneous, induced, transplanted and genetically engineered models [30], which are described below (Figure 1). The advantages (strengths) and disadvantages (limitations) of each model are summarized in Table 1.

2.1. Spontaneous models

Mammary tumors are the second most common type of spontaneous neoplasm in rats, after pituitary gland tumors [33]. Like in humans, this oncological condition is rare in male rodents and more frequent in intact females [34]. Several rat strains including August, Albany-Hooded, Copenhagen, Fisher, Lewis, Osborne-Mendel, Sprague-Dawley, Wistar and Wistar/Furth have been reported to spontaneously develop mammary tumors [35]. A study observed a range of incidence of spontaneous mammary tumors from 30 to 67% in Sprague-Dawley female rats [36]. Another factor influencing the development of spontaneous mammary tumors is the age. Older animals present a higher incidence when compared to younger animals, with the development of mammary tumors being rare before 18 months of age [37].

The literature regarding the development of spontaneous mammary tumors in mice is scarce and often controversial. The spontaneous mammary tumors of mice are associated with the mouse mammary tumor virus (MMTV) and their incidence is much lower than in rats [38].

Although spontaneous models are very interesting and useful, incidence rates are low, and the time required to obtain tumors, i.e. the latency period, is too long. To fulfil this gap, several rodent models of mammary carcinogenesis with decreased latency period and increased incidence have been developed.

2.2. Induced models

Chemically-induced models are the most commonly used rodent models for the study of mammary carcinogenesis. From an experimental point of view, chemical compounds

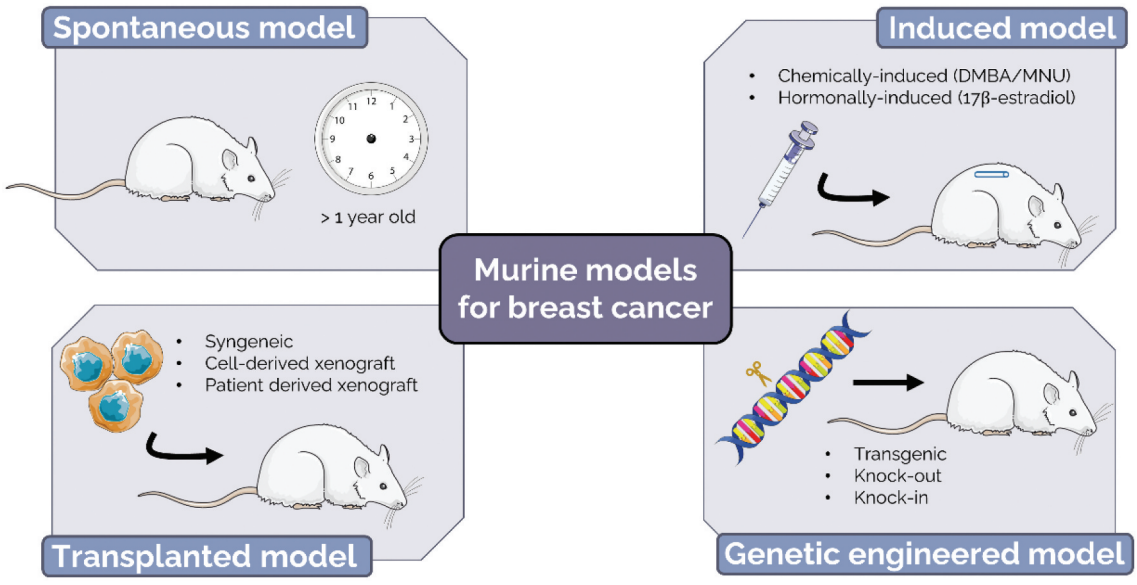

Figure 1. An overview of murine models of breast cancer used in cancer research. The figure was partly generated using Servier Medical Art, provided by Servier, licensed under a creative commons attribution 3.0 unported license.

Table 1. Advantages (strengths) and disadvantages (limitations) of spontaneous, induced, transplanted, and genetically engineered models of breast cancer [15,18,22,30–32].

Models		Advantages/Strengths	Disadvantages/Limitations
Spontaneous		Human-like tumorigenesis	Low incidence, long latency, extensive experimental protocols
Induced		High incidence rates and short latency	Lower induction rates, long development time and different incidence times
		More accurate predictions	
		Possibility of analyzing the stages of carcinogenesis and how they relate to environmental factors	Heterogeneous pathological characteristics
			Some biological characteristics can have an impact on the number of tumors, latency duration, and histological type
			Limited metastatic potential
Transplanted	Allografts	Multiple characterized cell lines	Transplanted cells not from human origin
		Rapid growth and metastasis	
		Immune-component microenvironment	
	Xenografts CDX	Can show primary tumor growth	Expensive, time consuming and multidisciplinary expertise needed
		Relatively homogeneous histological features	Inability to carry out preventive studies
		Can analyze all steps of the metastatic cascade	
	Xenografts PDX	Study of pharmacokinetics and distribution of drugs	Expensive, time consuming and multidisciplinary expertise needed
		Ability to serially expand therapy resistance tumors	Inability to carry out preventive studies
		Can analyze all steps of the metastatic cascade	Cannot mimic immune system and tumor-host interaction
Genetically engineered		Intact immune function with a complete microenvironment	Expensive, time consuming and the histology features differ from human breast tumors
		Human-like genetic alterations	
		Model the entire metastatic cascade and exert genetic control over metastasis	Sometimes long period of tumorigenesis
			Genetic breeding colony is necessary
		Study mechanisms and pathways of diseases in a complex organism enabling drug testing and development	Gene edition occurs in almost all mammary ductal epithelial cells
			Different inflammatory and desmoplastic response

CDX: cell line-derived xenograft; PDX: patient-derived xenograft.

are considered carcinogens when their administration induces a statistically significant increase in tumor incidence when compared with the control group [39]. *N*-methyl-*N*-nitrosourea (MNU) and 7,12-dimethylbenz[a]anthracene (DMBA) are the two carcinogens more commonly used to induce mammary carcinogenesis in rodents [35].

In 1961, Charles Brenton Huggins developed the first rat model of mammary cancer DMBA-induced [40]. Since then, models of mammary cancer chemically-induced have been widely used for breast cancer research [35,41]. MNU and DMBA may be administered intravenously, subcutaneously, intraperitoneally or intragastrically, and a single administration of these compounds leads to the development of mammary tumors in the span of a few weeks [42]. Both carcinogens promote the development of hormone receptor-positive tumors and the spectrum of induced lesions varies from adenomas, adenocarcinomas, tubular, papillary, cribriform or comedo carcinomas [35,43]. Despite this, looking to the previous studies performed by our research team in this field, we observed that MNU leads to the development of more aggressive mammary tumors when compared with those induced by DMBA [44]; and a higher number of mammary tumors were observed in the glands of the thoracic region and those of the right mammary chain, for both carcinogens [45,46].

The Sprague-Dawley and Wistar rat strains and the BALB/c and C57BL/6 mice strains are widely used as models of mammary cancer chemically-induced, as they are more

susceptible to carcinogens when compared with other strains. This susceptibility is particularly pronounced when these agents are administered around 50 days of age, coinciding with the animals' puberty and a heightened rate of cell division within the mammary gland [47]. The vulnerability of the rat mammary gland to carcinogens declines with age due to a decrease in the quantity of undifferentiated structures [48]. Due to similar reasons, when chemical carcinogens are administered after pregnancy or breastfeeding, tumor incidence is lower [48]. Although both carcinogens are effective for mammary cancer induction, the latency period is lower in the MNU-induced model when compared with the DMBA-induced model, because MNU is a direct alkylating agent, while DMBA is an indirect carcinogen [44,49].

Although mice are used for genetic analysis, rats have been used more frequently in toxicological research. This is partly because rats live longer than mice and develop a wider range of cancers that are morphologically comparable to those found in humans [50]. In addition, rats are free of MMTV and are more sensitive to chemical carcinogens and radiation than mice [51]. Although mice are less used as chemically induced models than rats, mammary carcinomas have been developed in mice using carcinogenic agents such as 3,4-benzopyrene, 3-methylcholanthrene, 1,2,5,6-dibenzanthracene, DMBA, and urethane. Chemically-induced mammary tumors in mice develop over a large latency period, and the induction requires several administrations [35]. Most mammary tumors chemically-induced in mice were classified as adenoacanthomas and type B adenocarcinomas [35].

In addition, mammary tumors can also be induced using hormones by introducing them in implants subcutaneously into animals or through the use of hormone injections [52,53]. Some hormones that are used include 17β-estradiol and medroxyprogesterone acetate [52,54]. This induction method requires specific strains, because not all strains will develop tumors, with the AC1 strain being the most often found in studies using this model [52,55,56].

Chemically-induced models can also be co-administered with hormones, such as estrogen and progesterone, to accelerate the progression of mammary tumors [57].

2.3. Transplanted models

Transplantation models are obtained by transplanting cancer cell lines or solid tumors from a donor. The first xenograft breast cancer model was described in 1962, when human breast cancer was heterotransplanted into an immunodeficient mouse [58]. Based on the source of the transplant, these models can be divided into cell-derived xenografts (CDX), patient-derived xenografts (PDX), or syngeneic models (also known as allograft models) [59]. When the tumor donor and host are from different species, they are classified as xenograft models. On the other hand, when the tumor donor and host are from the same species, they are classified as syngeneic models [60,61]. Both models can be classified as orthotopic or heterotopic, considering the implantation sites. Orthotopic models involve transplanting the tumor in its original site, whereas heterotopic (also known as ectopic) models consist of transplanting tumor material to a location other than their original site [62]. The immune state is a major issue in the development of transplanted models because the host animals must have a low immune system to ensure that they do not reject the implanted cells or tumor. Despite the disadvantages, these animal models are important tools for studying the behavior and growth of human cancer cell lines and tumors *in vivo* [33,63,64]. Animals can be classified according to their immune status as immunocompetent or immunocompromised [65]. Immunocompetent hosts have a complete immune system; i.e., they can produce a normal immune response upon exposure to an antigen. In contrast, immunodeficient animals refer to those that have defects in one or more immune components (such as T, B, NK cells) in the immune system. Examples of immunodeficient strains used and their immunological characteristics are shown in Table 2. There are also animals whose murine hematopoietic system has been replaced by human hematopoietic stem cells in the bone marrow to reconstitute the human immune system, avoiding the rejection of human-derived tumor by animals [65,66].

In these models, the time required for the appearance of tumors in animals varies according to the injection/transplantation protocols used, considering the strain, cell line, concentration tested and site of transplantation. The models commonly employed for breast cancer research are nude (athymic), severe combined immunodeficient (SCID), non-obese diabetic-severe combined immunodeficient (NOD-SCID), Rag-deficient (RAG), NOD/Shi-scid/γc-/- null (NOG), and NOD/SCID/γc-/- (NSG) mice strains [67]. Nude mice are the most commonly used to perform xenograft models. These animals received this name because they have a mutation on chromosome 11 called 'nude' that causes phenotypic and functional changes. They lack a functional thymus and, as a result, have a low number of mature T lymphocytes, which is critical to prevent cell or tissue rejection [68].

Although the mice are more frequently used than rats as transplanted models, there is also a nude rat strain (rnu/rnu), which possesses an autosomal recessive mutation known as *rnu*. It was backcrossed with several strains, and as a result, produced

Table 2. Immunodeficient mouse strains used in breast cancer research [67,71].

Mouse strain	Immunological features
Athymic Nude	Functional T-cell deficiency
RAG	Absence of functional T-cells and B cells deficiency
SCID	Absence of functional T-cells and B cells deficiency
NOD-SCID	Absence of functional T-cells and B cells deficiency; Absence of C5 complement; residual NK activity
NOG	Absence of functional T-cells and B cells deficiency; Absence of C5 complement; extremely low NK activity
NSG	Absence of functional T-cells and B cells deficiency; Absence of C5 complement; extremely low NK activity

Nk: natural killer; NOD-SCID: non-obese diabetic-severe combined immunodeficient; NOG: NOD/Shi-scid/γc-/- null; NSG: NOD/SCID/γc-/-; RAG: Rag-deficient; SCID: Severe combined immunodeficient.

many congenic strains characterized by congenital thymus absence and hairlessness [69]. MCF-7, MDA-MB-231 and 4T1 are the most used breast cancer cells lines. The number of cells injected in rodent models for breast cancer can vary widely from thousands to millions of cells depending on the study [22]. Phosphate-Buffered Saline (PBS) and Matrigel are the most common choices of solvent or vehicle for injecting cells into animals in research experiments [70].

In orthotopic model of breast cancer, breast cancer cells are transplanted into the mammary fat pad or mammary duct, while in heterotopic model breast cancer cells implantation occurs in another site such as subcutaneous, tail vein and left ventricular injection [67,71].

2.3.1. Syngeneic models

Syngeneic approaches use cells obtained from tumors developed in spontaneous or induced rodent models and insert them into host mice from the same inbred genetic background to avoid the need for immunocompromised host animals. The fact that tumor cells, microenvironment and host are from the same species is the main advantage of this model. Furthermore, because these models are immunocompetent, they may be utilized to investigate how the immune system is involved in tumor initiation, promotion, progression, and metastasis. The lack of heterogeneity and mutations that characterize human tumors is the main limitation of this model [72].

Several syngeneic models have been established using different mammary cancer cell lines obtained from mice, such as 4T1, EMT6, TM40, and D2A1 from BALB/c mice, E0771 from C57BL/6 mice and MVT1, 6DT1, and M6 from FVB mice. The BALB/c-derived 4T1 is a triple-negative cell line and the most common murine mammary cell line used in research as an orthotopic model. This model has the high metastatic capacity to lungs and lymph nodes, and well-vascularized nature of tumors as main advantageous characteristics [73–76].

2.3.2. Cell-derived xenograft

In this cancer model, cell lines are injected into immunedeficient mice. The cell-derived xenograft (CDX) model derived from different tumor cell lines, which confers unique characteristics to each model, such as histological features, molecular subtype and metastatic potential [77]. This model is commonly used to better understand cancer genetics and drug resistance mechanisms. Different breast cancer cell lines can be transplanted into mice to establish a CDX model, allowing the validation of target genes of interest as well as the metastasis process and therapeutic response. Inversely to the breast tumors' heterogeneity, this model develops relatively homogeneous tumors with loss of original cellular characteristics which constitutes a limitation. Due to selective pressure on cell culture *in vitro*, cancer cell lines tend to lose the heterogeneous features of the original tumor. These models are also unable to simulate the tumor microenvironment, because it cannot replicate the immune system's response, since this technique is usually performed in nude mice, which lack T-cell function, or other immunocompromised mice strains [78,79]. However, this model presents several advantages, namely its low cost when compared to PDX, high availability, high reproducibility and short establishment time [80].

As mentioned above, the most used cell lines in CDX models are MCF-7 (estrogen receptor-positive) and MDA-MB -231 (triple-negative). The transplantation of MDA-MB-231 cells results in a more invasive, metastatic, and experimentally reproducible model than MCF-7 cells [81,82]. Furthermore, using estrogen-dependent breast cancer cells (such as MCF-7) requires the introduction of additional supplements like estradiol [67,83]. In addition, cancer cell lines such as MDA-MB-231 and SUM149 can be injected into the tail vein of the mice to establish metastatic CDX models [59]. The direct implantation of human breast cancer cell lines into the mouse mammary fat pad results in a simulation of human breast cancer [84].

2.3.3. Patient-derived xenograft

The PDX model is obtained by transplanting the human patient tumor materials into immunocompromised mice. Tumor materials from patients might be either minced tissue or single-cell suspensions [85,86].

These models are of great interest as they are derived directly from tumor samples and have never been cultured *in vitro*. They are very close to patients in terms of biological behavior, such as gene expression profiles, intrinsic phenotypes, genomic alteration, metastatic potential, and drug response [87]. In addition, the PDX model and its corresponding patients showed similar responses to certain therapeutic treatments [86]. In contrast to CDX models, this model is more costly, has low take rates and requires more time to be established. Other limitations include the lack of an immune system and the impediment of studying the disease in its early stages. In return, it allows the mimicking of tumor microenvironment, maintaining histologic and genetic features, and using it as a metastatic model [80,88].

There are several studies using PDX models, however, not all specify the molecular tumor type. PDX models for the triple negative are the most used since it is the subtype with the greatest urgency for effective therapies. In addition, by being very aggressive, it shows high growth rates in animals [80,88]. Recently, there has been a preference for using tumor organoid lines in an attempt to overcome the challenge of studying tumor heterogeneity, the tumor microenvironment and drug screening within a clinically relevant context. These organoids, especially patient-derived organoids xenograft (PDOX), have gained prominence due to their ability to better recapitulate theses aspects. PDOX models have been established, and they have been demonstrated to mimic parental tumor features. PDOX can be derived directly by introducing patient-derived organoids into immunodeficient mice. They successfully preserve many key characteristics from the original tumor, including histopathological features, drug sensitivity and tumor invasiveness [86,89].

2.4. Genetically-modified animals

Genetically-modified models or genetically engineered models (GEMs) are organisms which genetic material have been altered by adding (transgenic), changing/modifying (knock-in), or

removing (knock-out) DNA sequences in a manner that does not ordinarily exist [33,90,91]. There are many benefits of using these animals, namely: the creation of recombinant products such as therapeutic antibodies and anticoagulants; a better understanding of the mechanisms underlying the human disease will enable the creation of effective and targeted treatments; production and analysis of safe and effective products for use on humans; method for researching diseases mechanisms in a complex organism [58,90]. GEMs can model several subtypes of breast cancer (e.g. luminal A/B, HER2-overexpressed and triple-negative) and are frequently used to investigate the effects of genetic alteration on mammary tumorigenesis, development, and metastatic progression [64,92–94].

First transgenic mice generated using MMTV was in 1984 by Philip Leder [95,96]. Nowadays, the most common transgenic animal model used in breast cancer research is the MMTV and the polyomavirus middle T-antigen (MMTV-PyMT) mouse model [97]. These genetic modifications cause the mouse to develop mammary tumors that closely resemble human breast tumors [59].

The ability to create genetically modified animals set new standards for the scientific community and allowed researchers to explore novel approaches to treat diseases, understand molecular mechanisms and create new drugs [33]. Despite this, there are several concerns about the welfare and health of this animal model, since we know that when genes are inserted or deleted, they could bring undesirable side effects caused by integration and expression of recombinant genes [98].

2.4.1. Humanized models

Humanized animal models are animals that have been genetically changed or designed to have certain human genes, tissues, or cells in order to replicate human illness situations more effectively [22]. In breast cancer research, humanized animal models are used to research many key features of human breast cancer development and progression [18]. They can help researchers to better understand disease development, progression, and find new and more effective therapeutic strategies [99]. In oncology, these models enable scientists to investigate a wide array of aspects, including tumor growth, invasion, metastasis and the interaction between cancer cells and the immune system [100]. Humanized animal models have various benefits for the study of breast cancer, namely the ability to test new drugs, elucidate tumor biology, and explore the significance of specific genes in cancer progression [18]. Nevertheless, it is crucial to emphasize that while these models provide valuable insights, none can precisely replicate the intricacy of clinical tumors [100]. To get a full understanding of breast cancer biology and prospective treatment methods, researchers often use a combination of various models and *in vitro* experiments [99].

3. Selecting the most suitable rodent model of breast cancer

Selecting the most suitable rodent model for breast cancer research can be a challenge, as there are various research scenarios and objectives to consider. It is important to consider the characteristics of the animal models available, the type of research carried out and the mechanisms of action of the therapies tested. Here, we provide a guidance for selecting the most adequate model for breast cancer research under various research scenarios/aims (Tables 3 and 4).

In tandem with the intricacies of selecting an appropriate rodent model for breast cancer research, it is also important to emphasize the integration of considerations for statistical power into this decision-making process. According to the 3 R's principle, particularly the reducement, experimental design should aim to minimize the number of animals used for ethical reasons [102]. However, it is equally ethically important to rigorously test experimental hypotheses, ensuring that an experiment uses a sufficient sample size to ensure reproducibility – a critical aspect of experimental design [103]. The calculation of sample size holds significance in animal studies. Opting for a smaller number of animals may result in overlooking significant differences present in the population, while selecting an excessive number may entail unnecessary costs, time, effort, resource use and ethical concerns [104,105]. Power analysis is a method used to calculate sample size and allows estimation based on the significance level and statistical power. This calculation should consider several variables, such as mortality rates, the number of groups, the standard deviation, the type 1 error, the power, the direction of the effect and the statistical test. This analysis can be performed using different available tools, such as various websites and software, facilitating researchers in conducting robust power analysis to estimate the minimum sample size required for an experiment, ensuring a reasonable likelihood of detecting an effect of a given size.

4. New trends in breast cancer research

Recent advances in breast cancer research have ushered in a new era of understanding this complex disease. As such, new cutting-edge approaches, including precise gene editing in rodent models using CRISPR/Cas9 [106], offer new insights into genetic alterations [107] and targeted therapies [107].

Alternative models have also been developed, like organ-on-a-chip systems. These microfluidic devices replicate the architecture and function of human organs and offer a unique approach for breast cancer research. They can be used to study tumor development, drug response, and the interactions between cancer cells and the microenvironment in a controlled and highly customizable setting [108].

Advanced imaging techniques, such as multiphoton microscopy [109], optical coherence tomography [110], and positron emission tomography [111], provide high-resolution images for noninvasive monitoring of tumor morphology, metabolism, and response to treatment. These techniques also complement animal experimentation and can be used for preclinical research to evaluate the efficacy and safety of the treatments in study [112].

Rodent models remain the gold standard for examining new therapeutic targets. More recently, mouse models with humanized hematopoietic systems have been used as valuable tools for preclinical research to evaluate the efficacy and safety of immunotherapies, as monotherapy or combination therapy, for triple-negative breast cancer [113,114]. Another

Table 3. Scenarios and recommendations, in the view of the authors, for choosing the most suitable animal model for various types of breast cancer research.

Scenarios/Aims	Recommendation(s)
Study genetic modifications	GEM
Role of carcinogens	Chemically-induced
Microenvironment or interactions between tumor cells and stromal components	CDX*
	PDX*
	GEM
	Syngeneic model
Role of immune system	PDX*
	Humanized model*
	Syngeneic model
	GEM
Metastasis study	CDX*
	Syngeneic model
Study subtypes	CDX*
	PDX*
Research focuses on testing novel breast cancer treatments or assessing treatment responses	GEM*
	PDX*
	AVATAR*
	CDX
	Chemically-induced
A limited research budget	Chemically-induced
Explore the genetic drivers of breast cancer subtypes	GEM
Carcinogenesis mechanism	Chemically-induced

* represents the most recommended model(s). CDX: cell line-derived xenograft; GEM: genetically engineered models; PDX: patient-derived xenograft.

Table 4. Animal model for different types of drugs. Adapted from [101] with permission of Elsevier.

Drug type	Animal model
Cytotoxic chemotherapy	Chemically-induced model
	Syngeneic model
	CDX
	PDX
	GEM
Molecular-targeted agents	Chemically-induced model
	Syngeneic model
	CDX
	PDX
	GEM
Immunotherapy	Syngeneic model
	GEM (Humanized)

CDX: cell line-derived xenograft; GEM: genetically engineered models; PDX: patient-derived xenograft.

option may be to develop mouse models using both human tumor xenograft models and genetic modifications to better understand the molecular mechanisms under breast cancer progression and metastasis [115]. Another intriguing breakthrough in rodent model research involves the concept of 'mouse avatars' [100]. In this approach, a segment of a patient's tumor is transplanted into immunodeficient mice, and subsequent generations of mice are used for drug testing, with the ultimate goal of developing a personalized patient therapy. The use of avatar models aligns with the principles of personalized medicine and has garnered considerable attention due to its potential to foster the development of personalized and successful cancer therapies [116]. Furthermore, it offers a valuable tool for evaluating drug responses, enabling the prediction of chemoresistance [117].

5. *In vivo* studies performed to assess the efficacy of antineoplastic drugs for breast cancer treatment

The contribution of animal models for scientific progress is incontestable. The use of rodents for modeling breast cancer is a feasible approach to determine the most sensitive stage of tumor development for the use of chemopreventive and/or therapeutic agents. Many animal models have been used in experimental works to address the prophylactic or therapeutic effects of several compounds in this oncological disease.

In Table 5 is displayed several studies on antineoplastic drugs, other pharmacological groups (nonsteroidal anti-inflammatory drugs and antibiotics), and natural compounds tested in the rodent models of mammary carcinogenesis, using different models. An electronic literature search was performed in the following scientific databases PubMed, ScienceDirect and Google Scholar, on 11 April 2023. Only full text articles published in English, in open access and indexed journals, between 2013 and 2023 were included. After reading the articles retrieved, we found thatmost studies used the Sprague-Dawley strain for rats and BALB/c strain for mice (Figure 2A,B). Transplanted models are the researchers' models of choice, with the xenograft models being the most used, whereas the chemically-induced models are the most used in the induced models with DMBA being the carcinogen of choice (Figure 2C). The combination of compounds, mainly an anti-neoplastic drug with a natural product, are the most investigated substances in current studies (Figure 2D). Looking to Table 5, we observed that tumor volume, latency and multiplicity and mortality rates as well as biochemical analyses to assess hepato- and nephrotoxicity are some parameters evaluated in rodent models to determine the efficacy and safety of drugs. For histological samples, the assessment of morphology, and histological grade as well as the determination of some biomarkers (e.g. VEGF, ki-67 and COX-2) are also key points used to evaluate the drugs. In addition, we observed that not all compounds have inhibitory effects on mammary tumors. We also concluded that doxorubicin is the most frequently found drug in the literature, possibly because it is already applied in clinical practice with good indicators, but still with high rates of cardiotoxicity. Studies have

Table 5. *In vivo* studies using different rodent models of mammary cancer to assess the efficacy of several therapeutic strategies.

Model			Drug	Dose, route of administration and duration	Therapeutic effects	Reference
Chemical carcinogenesis	♀ ACI rats	17β-estradiol	Resveratrol	50 mg subcutaneous pellet every other month for 8 months	Increased apoptosis and decreased DNA damage, cell migration, colony and mammosphere formation	[55]
	♀ ACI rats	17β-estradiol	Tochopherol	0.3% on diet for 1,3,7 and 14 days	Decreased nitrosative, oxidative stress markers, nitrotyrosine and 8-oxo-dG	[56]
	♀ Albino rats	DMBA	*Nigella sativa*, Thymoquinone	1/5 and 10 mg/kg, gavage, 3 times/week, for 4 months	Inhibited tumor growth	[118]
	♀ Holtzman rats	DMBA	*Piper aduncum*	50/150 and 300 mg/kg/(capsules), p.o.	Decreased mammary carcinogenesis and lymph node metastasis	[119]
	♀ Sprague-Dawley rats	DMBA	2,2′-diphenyl-3,3′-diindolylmethane	5 mg/kg, gavage, each two days for 21 days	Reduced tumor growth	[120]
	♀ Sprague-Dawley rats	DMBA	3-carbamoyl-2,2,5,5-tetramethylpyrroline-1-oxyl	10 mg/kg, i.p., 14 days	Reduced tumor volume and rate	[121]
	♀ Sprague-Dawley rats	DMBA	Allyl isothiocyanate	10/20 and 40 mg/kg, 16 weeks	Decreased number, volume, and incidence of tumors	[122].
	♀ Sprague-Dawley rats	DMBA	Asiaticoside	200 µg/animal, i.p., 2 weeks before and 8 weeks after DMBA administration or 5 weeks after DMBA administration	Enhanced anti-tumor activity	[123]
	♀ Sprague-Dawley rats	DMBA	Berberine	50 mg/kg, thrice weekly from 1 to 12 weeks	Effective against ductal carcinoma and invasive carcinoma	[124]
	♀ Sprague-Dawley rats	DMBA	*Chlorella pyrenoidosa*	3 and 30% in diet (w/w) for 14 weeks	Suppressed tumor frequency and increased tumor latency; increased caspase-7 expression and decreased VEGF-2 expression	[125]
	♀ Sprague-Dawley rats	DMBA	Cisplatin, Nordihydroguaiaretic acid	7.5 mg/kg, i.p., single dose +10 mg/kg	Reduced tumor volume and ameliorated nephrotoxicity effects	[126]
	♀ Sprague-Dawley rats	DMBA	Cloudy apple juice	10 mL/kg, gavage, 28 days before DMBA administration	Decreased blood levels, biochemical liver, and kidney markers	[127]
	♀ Sprague-Dawley rats	DMBA	Doxorubicin (Thermo/pH-responsive magnetic nanoparticles)	2 mg/kg/48 h, i.p., for 12 days	Reduced tumor volume and Ki-67 proliferation index and increased survival rate	[128]
	♀ Sprague-Dawley rats	DMBA	Folic acid	5/8 and 10 mg/kg diet, diet supplementation, for 12 weeks	Promoted tumor progression	[129]
	♀ Sprague-Dawley rats	DMBA	*Ganoderma lucidum*	500 mg/kg, gavage, for 16 weeks	Potent chemopreventive agent	[130]
	♀ Sprague-Dawley rats	DMBA	Isoflavone	100/500 or 1000 mg/kg diet, diet supplementation, for 24 weeks	Decreased tumor incidence, mean number of tumors per animals and increased tumor latency	[131]
	♀ Sprague-Dawley rats	DMBA	Methyl-25-hydroxy-3-oxoo-lean-12-en-28-oate	0,8/1.2 and 1.6 mg/kg, gavage, 3 times/week for 18 weeks	Inhibited mammary carcinogenesis	[132]
	♀ Sprague-Dawley rats	DMBA	Paclitaxel, *Eruca sativa* seeds	20 mg/kg/week Paclitaxel encapsulated liposome and 500 mg/kg/week *Eruca sativa* seeds, 4 weeks	Reduced inflammation	[133]
	♀ Sprague-Dawley rats	DMBA	Resveratrol, Copper supplementation	0.2 mg/kg resveratrol, gavage +42.6 mg Cu/kg copper, food gavage	Decreased iron and copper serum levels	[134]
	♀ Sprague-Dawley rats	DMBA	Shemamruthaa	400 mg/kg/day, gavage, for 14 days	Reduced lipid peroxidation, tumor multiplicity and tumor volume	[135]
	♀ Sprague-Dawley rats	DMBA	Simvastatin	20 and 40 mg/kg, gavage, for 14 days	Reduced tumor growth	[136]
	♀ Sprague-Dawley rats	DMBA	Spirulina	1% Spirulina mixed on standard diet	Spirulina reduced breast tumors incidence from 87 to 13%; reduced Ki-67 and estrogen α.	[137]
	♀ Sprague-Dawley rats	DMBA	Tamoxifen, Quercetin (loaded in Poly (lactic-co-glycolic acid))	3 mg/kg tamoxifen, gavage, 3 days and 3 mg/kg +6 mg/kg tamoxifen + quercetin, gavage, 3 days	Reduced tumor growth and angiogenesis	[138]
	♀ Sprague-Dawley rats	DMBA	Tangeretin	50 mg/kg, p.o.	Tangeretin administration was beneficial against DMBA-induced oxidative stress	[139]

(*Continued*)

Table 5. (Continued).

	Model		Drug	Dose, route of administration and duration	Therapeutic effects	Reference
	♀ Sprague-Dawley rats	DMBA	Taurine	100 mg/kg, gavage, for 5 weeks	Efficient as chemotherapeutic agent	[140]
	♀ Sprague-Dawley rats	DMBA	Taurine	3% of taurine given freely on water for 16 weeks	Reduced induced breast cancer from 80 to 40%	[141]
	♀ Sprague-Dawley rats	DMBA	*Trianthema portulacastrum*	50/100 and 200 mg/kg, diet supplementation, 18 weeks (2 weeks before and 16 weeks after DMBA administration)	Reduced inflammation and suppressed tumor development	[142]
	♀ Sprague-Dawley rats	DMBA	Vanadium, Fish oil	0.5 ppm vanadium, drinking water, 6 weeks +0.5 mL/day fish oil, gavage, for 6 weeks + (Vanadium + fish oil drinking water, 0.5 ppm + gavage, 0.5 mL/day, 6 weeks)	Inhibited mammary tumor growth; the combination was more effective	[143]
	♀ Sprague-Dawley rats	MNU	Celecoxib	1.67 g/kg (0.167%), p.o., in diet for 16 weeks	Reduced tumor frequency, prolonged tumor latency and tumor multiplicity	[144]
	♀ Sprague-Dawley rats	MNU	Doxorubicin, Iodine	4–16 mg/kg, i.p., 1 day + 4–16 mg/kg, i.p., 1 day + 0.05% drinking water, 7 days	Iodine can be used with doxorubicin in cancer therapy	[145]
	♀ Sprague-Dawley rats	MNU	Methotrexate, Curcumin	5 mg methotrexate +2.5 mg curcumin, injection, once a week for 4 weeks	Synergistic effect on inhibiting cancer progression	[146]
	♀ Sprague-Dawley rats	MNU	Methotrexate (loaded in chitosan nanoparticles)	5 mg/kg methotrexate (loaded in chitosan nanoparticles), i.v. tail vein, 5 weeks	Nanoparticles reduced tumor volume compared with free methotrexate	[147]
	♀ Sprague-Dawley rats	MNU	Pitavastatin, Melatonin	10 mg/kg pitavastatin, diet +2.1 mg/kg melatonin, drinking water	Pitavastatin + melatonin decreased tumor frequency, volume, and lengthened tumor latency	[148]
	♀ Sprague-Dawley rats	MNU	Tamoxifen, Tamoxifen (loaded in polymetric micelles)	5/7.5/10 mg/kg tamoxifen + 5/7.5/10 mg/kg tamoxifen loaded polymetric micelles, p.o., once in 3 days for 60 days	Tamoxifen loaded polymetric micelles are more benign than free tamoxifen treated	[149]
	♀ Sprague-Dawley rats	MPA-accelerated DMBA	Apigenin	Diet supplementation (0.02%, 0.1%, and 0.5%)	Promoted tumor development	[150]
	♀ Wistar rats	DMBA	Celecoxib, Fish oil	20 mg/kg celecoxib, 20 for 7 days +0.5 ml Fish oil, for 7 days	Upregulated Bax, Faz, Faz L. and Caspase-8; decreased Bcl-2 levels	[151]
	♀ Wistar rats	DMBA	*Crateva adansonii*	75 or 300 mg/kg, gavage, 12 weeks	Reduced tumor burden, weight, and volume	[152].
	♀ Wistar rats	DMBA	L-nitro arginine methyl ester	30 mg/kg, each 3rd day for 5 weeks	Decreased tumor histological grade from grade III to grade II; delayed tumor formation	[153]
	♀ Wistar rats	DMBA	Vincristine, Myricetin	500 µg/kg vincristine, i.p., once/week, for 4 weeks + 50/100 and 200 mg/kg myricetin, gavage, every day for 16 weeks	Each drug inhibited mammary carcinogenesis	[154]
Genetically modified	♀ C3(1)-SV40 Tag mice	Transgenic mouse model	Tamoxifen, Genistein	250 mg/kg GE diet supplementation + TAM, subcutaneous implant, 3 weeks	Increased tumor latency and prevented tumor development	[155]
Syngeneic	♀ Albino mice	Ehrlich Ascites Carcinoma	Doxorubicin, Thymoquinone, highly purified polysaccharide polymer	5 mg/kg doxorubicin +3 mg/mouse thymoquinone +100 µl/mouse highly purified polysaccharide polymer, subcutaneous injection, on the 12th, 19th and 26th weeks	Reduced tumor volume and Bcl-2	[156]
	♀ BALB/c mice	4T1	6-pentadecyl salicylic acid, Taxol)	2 mg/kg, i.v., for 21 days	Reduced tumor growth and metastasis and increased survival rate	[157]
	♀ BALB/c mice	4T1	6-pentadecyl salicylic acid Taxol)	2 mg/kg, i.v., for 21 days	Reduced tumor growth and metastasis and increased survival rate	[158]
	♀ BALB/c mice	4T1	Carboplatin	100 mg/kg, i.p., 3 cycles every 7 days for 26 days	Decreased mitotic and apoptotic index and vascularization	[159]
	♀ BALB/c mice	4T1	Centchroman, Genistein	10 mg/kg of centchroman +200 mg/kg of genistein, gavage, 3 times/week for 3 weeks	Reduced tumor growth and reduce mortality rate	[160]
	♀ BALB/c mice	4T1	Cisplatin Prodrug-Conjugated Gold Nanocluster	1 mg/kg, i.v., 3 times	Inhibited tumor growth and lung metastasis	[161]
	♀ BALB/c mice	4T1	Cordyceps sinensis	100 and 200 mg/kg, i.p., 14 days	Inhibited tumor growth	[162]

(*Continued*)

Table 5. (Continued).

Model		Drug	Dose, route of administration and duration	Therapeutic effects	Reference
♀ BALB/c mice	4T1	Docetaxel-linoleic acid conjugate (loaded in lipid emulsions)	10 mg/kg, i.v. tail vein, 3 days for 4 weeks	Reduced tumor volume	[163]
♀ BALB/c mice	4T1	Doxorubicin,4,4′ -Dithiodibutyric acid-hyaluronic acid-3-minophenyl boronic acid monohydrate	5 mg/kg, i.v. tail vein, 30 days	No inhibition effects observed	[164]
♀ BALB/c mice	4T1	Doxorubicin (loaded with reovirus)	5×10^{8} PFU, delivered intratumorally, single dose	Reduced tumor burden and metastasis	[165]
♀ BALB/c mice	4T1	Doxorubicin (loaded mixed micelles)	2 mg/kg, i.v., single dose	Anti-tumor effects and protective cardiotoxicity	[166]
♀ BALB/c mice	4T1	*Solanum nigrum*	250/500 mg/kg, p.o., 10 days	Inhibited tumor volume and weight; Increased lymphocytes cells	[167]
♀ BALB/c mice	TS/A	Horse-Spleen Ferritin	90 mg/kg HoS-ferritin, i.v., single dose	Reduced tumor volume	[168]
♀ BALB/c mice	4T1	Carboplatin, Thalidomide	100 mg/kg carboplatin, i.p., 3 cycles + Thalidomide 150 mg/kg, gavage, 7 days	Synergistic suppression in tumor progression; reduced number of lung metastasis	[169]
♀ BALB/c mice	Ehrlich Ascites Carcinoma	Docetaxel, Thymoquinone	2 mg/kg docetaxel +4 mg/kg thymoquinone, i.v. tail vein	Enhanced antitumor effect	[170]
♀ BALB/c mice	murine macrophage Raw264.7 and 4T1	Paclitaxel- Human serum albumin and nanoparticles; Paclitaxel-Palmitic acid-modified human serum albumin nanoparticles	7.5 mg/kg PTX-HSA NPs +7.5 mg/kg PTX-PSA NPs, i. v. tail vein, day 6 and 11	Decreased vascular perfusion and liver metastasis	[171]
♀ C57BL/6 mice	E0771	Doxorubicin, Doxorubicin-loaded polynanoparticles	10 mg/kg doxorubicin and doxorubicin-loaded polynanoparticles, every 3 days for a total of 5 doses	Doxorubicin-loaded polynanoparticles reduced tumor volume in compared to free doxorubicin	[172]
♀ FVB mice	A17	Gold(I) azolate/phosphane compounds	Gold compounds, i.p., 12 mg/kg, 4 times every 3 days	Gold compounds induced apoptosis	[173]
♀ FVB/NCrl mice	A17	Ruthenium compounds [Ru (p-cymene) (bis (3,5-dimethylpyrazol-1-yl) methane)Cl]	52.4 mg/kg, every 3 days for 39 days	Anti-tumor effects and reduced number of tumor-infiltrating regulatory T cells	[174]
♀ SHR rats	SST-2	Doxorubicin, Mito-Tempol, Dexrazoxane	Doxorubicin (i.v., 10 mg/kg), Mito-T (i.p., 5 and 25 mg/kg), Dexrazoxane (i.p., 50 mg/kg), Mito-T + dexrazoxane (i.p., 5 and 25 mg/kg +50 mg/kg)	Doxorubicin, Mito-T and Dexrazoxane inhibit mammary carcinogenesis and doxorubicin-induced cardiomyopathy was improved	[175]
♀ Swiss mice	Ehrlich Ascites Carcinoma	*Capsicum annuum* L. cv Magali (CAP), Methotrexate	50/100 and 150 mg/kg CAP, gavage, 21 days +2.5 mg/kg methotrexate, every 5 days for 21 days	Reduced tumor growth, gene expression of vascular endothelial growth factor, vessel areas of the tumors and induces necrosis. Increases IL-6 in tumor and regulate inflammation and angiogenesis	[176]
♀ Swiss mice	Ehrlich Ascites Carcinoma	Methotrexate, Paclitaxel	2.5 mg/kg methotrexate, i.p., every 3 days +0.6 mg/kg paclitaxel, weekly	Methotrexate and Paclitaxel were effective in reducing tumor growth	[177]
♂ Swiss albino mice	Ehrlich Ascites Carcinoma	Diosgenin (loaded in poly lactide-co-glycolide)	10 mg/kg, i.v. tail vein, every 2 days for 28 days	Inhibited tumor growth and angiogenesis	[178]
BALB/c mice	4T1	GK-1	10/50 or 100 µg of GK-1 per mouse, i.v., 3 times in 24 days	Slowed primary tumor growth and lung metastasis development	[179]
BALB/c mice	4T1	Cisplatin, β-2-himachalen-6-ol	2.5 mg/kg cisplatin, i.p., once a week +25 mg/kg β-2-himachalen-6-ol, twice a week	Less incidence of primary and metastatic tumor/inflammation	[180]
BALB/c mice	4T1	Docetaxel (loaded in folate-conjugated dextran-poly lactide-co-glycolide)	10 mg/kg, i.v. tail vein, single dose	Inhibited tumor effects	[181]
BALB/c mice	4T1	Paclitaxel, Rottlerin	5 mg/kg paclitaxel +20 mg/kg rottlerin, i.p., each alternative day for 2 weeks	Increase of pro-apoptotic potential; Reduce tumor growth and metastasis	[182]
Wistar Furth rats	MT-450	Delphinidin	1.18×10^{-5} mol, gavage, 28 days	Promoted tumor growth and metastasis	[183]

(*Continued*)

Table 5. (Continued).

Model			Drug	Dose, route of administration and duration	Therapeutic effects	Reference
Xenograft	♀ BALB/c nu/nu mice	MDA-MB-231	Iodine	0.0025%, p.o., 3 weeks	Inhibited tumor growth	[184]
	♀ BALB/c nu/nu mice	MCF-7 and MDA-MB-231	Thioalbamide	0.5 mg/kg, i.p., 3 times/week	Inhibited tumor growth and reduces tumor dissemination	[185]
	♀ BALB/c mice	MCF-7	Chitosan, ursolic acid and folate (loaded in nanoparticles)	12.5 mg/kg/day, i.p., 9 times	Reduced breast cancer burden	[186]
	♀ BALB/c mice	MCF-7	Geldanamycin	14.3 and 28.6 mg/kg, i.v., on day 1, 4, 7 and 11	Reduced tumor progression and hepatotoxicity	[187]
	♀ BALB/c mice	MDA-MB-231	Curcumin	50/200 μg/kg, i.p., every other day for 4 weeks	Growth inhibition and induced apoptosis	[188]
	♀ BALB/c mice	MDA-MB-231	Docetaxel (encapsulated lipid polymer hybrid nanoparticles)	10 mg/kg, i.v., single dose	Reduced tumor burden and cytokines in serum	[189]
	♀ BALB/c mice	MDA-MB-231	Thymoquinone	4 or 8 mg/kg thymoquinone, i.p., 6 days/week +2.5 mg/kg doxorrubicin, i.p., once/week and thymoquinone (4 mg/kg, i.p., 6 days/week) + doxorrubicin (2.5 mg/kg, i.p., once/week)	TQ suppressed tumor growth, especially combined with DOX	[190]
	♀ BALB/c mice	MDA-MB-231	Paclitaxel, Curcumin [encapsulated in amphiphilic di-block copolymer poly(ethylene glycol)-block-poly (lactide-co-glycolide)]	6 mg/kg paclitaxel +4 mg/kg curcumin, subcutaneous route, single dose for 12 days	Reduced tumor volume and tumor progression	[191]
	♀ BALB/c mice	MCF-7 and MDA-MB-453	*Euphorbia fischeriana* Steud., *Ziziphus jujuba* Mill.	2.5/5.0/10.0 g/kg, i.g. single dose for 4 or 8 weeks	Reduced tumor weight and rate. Increased level of ALT, AST, Cr and BUN, and increased the hepatic and renal toxicity	[192]
	♀ C57BL/6J mice	MCF-7 and T-47D	Gemcitabine, Cisplatin	1/0.05 and 4/0.2 mg/kg Gemcitabine+cisplatin, i.p., 2 times with interval of 48 h, one day after cells' implantation	Reduced tumor volume and rate	[193]
	♀ FVB mice	BT474	Parecoxib, Sufentanil	5 mg/kg (with 1 μg/kg of sufentanil)	Inhibited tumor growth and metastasis	[194]
	♀ NOD.Cg-Prkdc scid Il2rg mice	AXF401 and MAXF57	Docosahexaenoic acid, Docetaxel	3.8 and 1.6 w/w in diet for 49 days, 5 mg/kg Docetaxel i.p. twice weekly	Reduced tumor growth and increased necrotic tissue	[195]
	♀ NOD/SCID mice	MDA-MB-231	Celecoxib	30 mg/kg, gavage, daily for 30 days	Reduced tumor volume and weight	[196]
	♀ NOD/SCID mice	SUM-159	Resveratrol	100 mg/kg, i.v., daily for 2 weeks	Inhibited tumor growth	[197]
	♀ Nu/Nu mice	MDA-MB-231	Tamoxifen, Genistein	250 mg/kg GE, diet supplementation + TAM, subcutaneous implant, 3 weeks	GE and GE+TAM promoted tumor suppression	[155]
	♀ Nu/nu mice	T-47D and BT-474	Cannabis, Tamoxifen, Cisplatin, Lapatinib	45 mg/kg cannabis, 3 days/week +2.5 mg/kg tamoxifen, i.p., 3 days/week +3 mg/kg cisplatin, i.p., 3 days/week +100 mg/kg lapatinib	Combination of cannabis with tamoxifen, cisplatin or lapatinib produced either positive or negative effects	[198]
	♀ SCID mice	MDA-MB-231	Tilarginine, Docetaxel	80 or 200 mg/kg L-NMMA, i.p., daily +20 mg/kg docetaxel, i.p.	Decreased tumor growth and enhanced survival rate	[199]
	♀ SCID mice	SUM-149	*Ganoderma lucidum*	28 mg/kg, gavage, for 13 weeks	Reduced tumor growth and weight	[200]
	♀ Wistar rats	MBCDF-T	Dovitinib, Calcitriol	20 mg/kg dovitini, i.p., 2 times/week +0.25/100 μL calcitriol, i.p., once a week	Inhibited tumor growth, decreased endothelial cell growth, tumor-vessel density and VEGFR2 expression	[201]
	BALB/c nu/nu mice	MDA-MB-231	Paclitaxel (loaded in nanoparticles – CD133NPs)	40 mg/kg/dose, i.v., on days 0 and 7	Inhibited tumor growth	[202]
	BALB/c nu/nu mice	MCF-7/ADR	Taxol (loaded in super-antiresistant micelles)	10 mg/kg taxol, i.v. +10 mg/kg super-antiresistant Paclitaxel micelles, i.v. +10 mg/kg taxol, p.o. +10 mg/kg super-antiresistant Paclitaxel micelles, p.o., once every 3 days 32 days	Super-antiresistant paclitaxel inhibited tumor growth	[203]
	Nu/nu mice	MCF-7/ADR	Doxorubicin (loaded poly (2-(diisopropylamino)ethyl methacrylate) micelles)	5.0 mg/kg, i.v., 1 injection every 3 days for 5 days	Inhibited tumor growth more efficiently	[204]
	Sprague-Dawley rats	T-47D and BT-474	Medroxyprogesterone acetate, 3-(5′-hydroxymethyl-2′-furyl) -1-benzylindazole (YC-1)	Medroxyprogesterone acetate pellets + YC-1 (10 mg/60-day release + YC (i.p., 600 μg))	Reduced tumor volume and size	[205]

♀: female; DMBA: 7,12-dimethylbenz[a]anthracene; i.g.: intragastric; i.p.: intraperitoneal injection; i.v.: intravenous injection; MNU; N-methyl-N-nitrosourea; PFU: plaque forming units; p.o.: oral administration.

advanced in this direction, to reduce the side effects caused by doxorubicin without losing its efficacy [145,175]. This drug is also studied with different forms of delivery to increase its efficacy and targeting [128,165,166]. After doxorubicin, paclitaxel and curcumin are the most widely used compounds.

6. Expert opinion

Breast cancer is a highly heterogeneous disease with varying etiology and pathology. Over the last decades, its incidence has been increasing, which may be attributed to a change in lifestyles that includes well-known risk factors, such as smoking, alcohol consumption and obesity. Several research teams have addressed the effects of westernization of lifestyle in breast cancer development and progression. For this, the researchers have evaluated the effects of exercise training on breast cancer by submitting animals to different types of exercise, with different durations and intensities. They have also addressed the effects of lifestyle by feeding animals with western diets. Our research team is one of those that has employed their efforts in this field and performed an experiment addressing the effects of lifelong moderate exercise training on BC development in which the animals were training on a treadmill 1h per day, at a velocity of 20 m/min, 5 days/week, for 35 consecutive weeks. After this, we observed that an active lifestyle reduced the number and malignancy of mammary tumors [206]. More recently, we developed a new protocol addressing the interplay between diet and exercise on mammary cancer development. In this experiment, the animals were trained in a ladder and fed with a western diet with 60% of total calories coming from fat. The animals were trained 3 days/week for 18 consecutive weeks, by climbing a 1 m-high homemade ladder. For each session of exercise, the animals made 4–8 climbs and 8–12 dynamic movements for each climb. The results of this protocol are still under analysis.

The promotion of screening initiatives has contributed for an earlier detection and, consequently, an improved prognosis, but mortality rates remain high and there is no effective therapy to increase the survival rate. Surgery, systemic chemotherapy, and radiotherapy are established as commonly used practices in the treatment of breast cancer, but these have several serious side effects and are not always successful. Therefore, research should continue to focus on increasing the effectiveness of treatments, while lowering their negative effects on the patient's quality of life. Understanding the molecular mechanisms of breast cancer and drug interactions has been made possible using models that resemble their human counterparts, namely cell culture and animal experimentation.

The use of *in vivo* models plays a crucial role in breast cancer drug discovery and the development of novel approaches. Compared to cell culture, animal models contribute for a better understanding of the complex interactions between cancer cells and their surroundings, namely the tumor microenvironment. Rodent models are widely used in breast cancer research because they are easy to manipulate and provide a controlled environment for studying this disease, being less restrained by ethical issues when compared to other animals, like dogs, cats, pigs, and non-human primates. Researchers have a wide range of breast cancer *in vivo* rodent models available for use, including spontaneous, chemically induced, transplanted and GEMs. Each model has its own advantages and disadvantages, and should be chosen according to the work plan, purpose, budget and equipment.

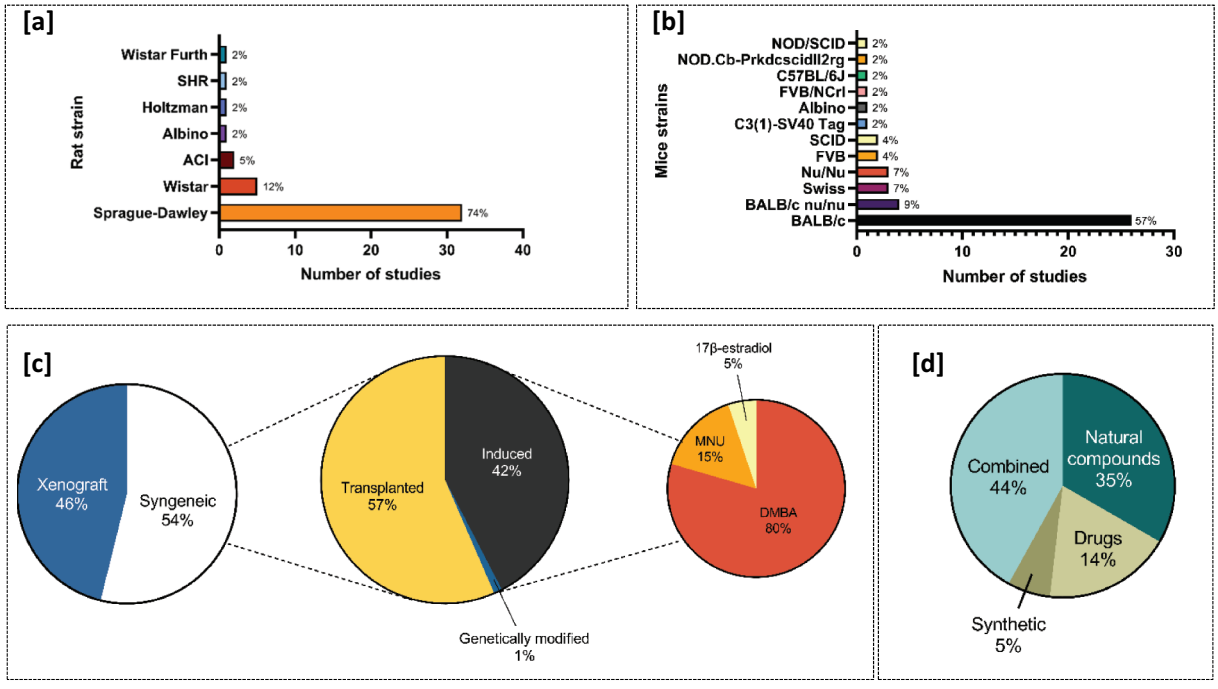

Figure 2. Schematic representation of the data from Table 1: [a] rat and [b] mice strains used in breast cancer research, [c] methods of induction and [d] compounds used in the studies.

Spontaneous models can provide insights into the role of specific genetic alterations in breast cancer development and progression. Chemically induced models provide information about the mechanisms of carcinogenesis and can be useful for the evaluation of chemopreventive agents' efficacy, being considered a less expensive alternative. Transplanted models can be used to study tumor growth, metastasis, and the effects of various treatments on tumor progression. GEMs involve the manipulation of specific genes in mice to induce the development of tumors, enabling researchers to study the role of specific genetic alterations, providing valuable insights into the molecular mechanisms underlying the disease.

Despite their advantages, *in vivo* models also have some limitations. For instance, patient-derived xenograft models may not fully recapitulate the human immune system's response to the cancer cells, and GEMs may not always accurately represent the genetic complexity of human breast cancer. Even though no model, either *in vivo* or *in vitro* can fully replicate the human disease and that no tumor is the same, these models are nonetheless able to provide the necessary information for drug screening, increasing the likelihood of successful translation of preclinical findings to clinical trials.

Due to the broader knowledge of the various molecular subtypes of breast cancer (luminal A and B, HER2 enriched and triple-negative), research and therapeutic approaches have focused on this direction. Endocrine therapy and HER2-targeted therapy, as well as immunotherapies, are emerging therapies that have been widely investigated. Giving that these therapies are designed to target specific molecular subtypes, researchers often select transplant models to ensure the precise subtypes in the study. Furthermore, PDX models may be used to test the efficacy of specific drugs on patients' tumors before treatment. In this way, PDX models are increasingly sought after, but the choice of recipient rodent strains, the use of hormonal supplements and the implantation site are factors that can introduce variability in experimental outcomes.

The establishment of standardized protocols plays a pivotal role in enabling high reproducibility when using these models, while transparency in published research is equally indispensable. Beyond the above-mentioned factors (strain, hormonal supplements, and implantation site), it is crucial to provide information on the culture method, number of passages of the cell line, concentration, vehicle used, monoculture or co-culture, 2D or 3D cultures (including spheroids or organoids). Furthermore, the disclosure of reagents and equipment used is essential, as these elements can be a factor contributing to protocol variations. Embracing dissemination and transparency in published research not only benefits the scientific community, ultimately reducing variations among research teams and enhancing the robustness of research outcomes. While chemically-induced models may seem outdated, their continued prevalence can be attributed to well-established protocols (specifying factors like dosage, administration route and age). Furthermore, they are easy to implement, and there are several carcinogens available on the market. These protocols ensure a high induction rate and mammary tumors closely resemble those found in humans in terms of histology, hormone dependence, expression of estrogen receptors and genetic alterations. As a result, researchers can effectively study the different stages of breast carcinogenesis, encompassing benign, pre-neoplastic and neoplastic lesions [33].

Recent discussions in Europe regarding the potential ban on animal experimentation for research purposes have prompted questions about the future of using animal models in breast cancer research, impacting both the pharmaceutical industry and academia. Traditionally, academia has relied on rodent models for fundamental research, while the pharmaceutical industry employs these models for drug development and testing purposes. Consequently, a ban would impact these sectors differently. Academics might face challenges in conducting fundamental research, potentially hindering discoveries. Conversely, the pharmaceutical industry, focused on drug development, may need to adapt by investing more in alternative approaches such as *in vitro* or computational modeling. These alternatives, though less complex than living organisms, may require additional refinement. These potential changes underscore the need for ongoing efforts to improve animal experimentation, with careful consideration of animal welfare. This concerted effort is not only pivotal for the refinement of scientific practices but also serves to reshape societal perceptions. The establishment of humane endpoints is essential to minimize animal suffering and ensure a responsible use of these animals.

Overall, rodent models of breast cancer have been invaluable tools in advancing our understanding of this disease, along with many others. They allow the development of novel therapeutic agents used as monotherapies or in combination with conventional chemotherapeutic agents. In addition, the use of genome editing tools, as well as advanced imaging techniques that allow for more refined protocols, could improve the accuracy of the collected data. The future of breast cancer research seems to be shifting toward more personalized approaches, which will lead to more targeted therapies, adapted to specific breast cancer subtypes and genetic profiles. To this end, researchers have tended to make greater use of PDXs and GEMs that closely mimic the tumors of individual patients. It is essential to recognize their limitations and continue to refine and improve these models to ensure their relevance and applicability in the ongoing fight against breast cancer, without compromising the ethical concerns and animal welfare.

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ORCID

Tiago Ferreira http://orcid.org/0000-0002-6652-9770
Tiago Azevedo http://orcid.org/0000-0002-3030-4510
Jessica Silva http://orcid.org/0000-0001-7698-1595
Ana I. Faustino-Rocha http://orcid.org/0000-0001-5572-6317
Paula A. Oliveira http://orcid.org/0000-0001-9519-4044

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