Mouse Models of Breast Cancer: Platforms for Discovering Precision Imaging Diagnostics and Future Cancer Medicine

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Representing an enormous health care and socioeconomic challenge, breast cancer is the second most common cancer in the world and the second most common cause of cancer-related death. Although many of the challenges associated with preventing, treating, and ultimately curing breast cancer are addressable in the laboratory, successful translation of groundbreaking research to clinical populations remains an important barrier. Particularly when compared with research on other types of solid tumors, breast cancer research is hampered by a lack of tractable in vivo model systems that accurately recapitulate the relevant clinical features of the disease. A primary objective of this article was to provide a generalizable overview of the types of in vivo model systems, with an emphasis primarily on murine models, that are widely deployed in preclinical breast cancer research. Major opportunities to advance precision cancer medicine facilitated by molecular imaging of preclinical breast cancer models are discussed.

Key Words: animal imaging; molecular imaging; breast cancer; coclinical trials; mouse models; precision medicine

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Breast cancer is the second most common cancer in the world, with an estimated 1.67 million new cases diagnosed in 2012, and the second most common cause of cancer-related death (*1*). In the United States alone, the American Cancer Society estimates diagnoses of more than 231,000 new cases of invasive breast cancer among women and approximately 2,350 new cases among men in 2015 (*2*). Uniquely, the term "breast cancer" does not reflect a single disease; rather, breast cancer should be thought of as a repertoire of related diseases classifiable into distinct subtypes, each portending distinct prognoses and potentially actionable phenotypic, molecular, or genetic characteristics. Although targeting certain molecular vulnerabilities inherent in specific breast cancer subtypes has improved clinical outcomes in a limited number of patients, a sobering reality is that more than 40,000 individuals in the United States will die from this disease in 2015 (*2*); this information underscores the numerous challenges that still remain in the clinical care of individuals with this disease.

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Although many of the challenges associated with preventing, treating, and ultimately curing breast cancer are addressable in the laboratory, successful translation of groundbreaking laboratory research to clinical populations remains an important barrier. Particularly when compared with research on other types of solid tumors, breast cancer research is hampered by a lack of tractable in vivo model systems that accurately recapitulate the relevant clinical features of the disease. Although certain models necessarily will be highlighted as a consequence of illuminating examples and opportunities, more exhaustive catalogs of previously described models are reviewed in several suggested references (*3–7*). Here, a generalizable overview of the types of in vivo model systems, with an emphasis primarily on murine models that are widely deployed in preclinical breast cancer research, is provided; this overview encompasses the specific relationship of the models with the clinical disease and how imaging within the context of the models might be exploited to maximize translational gains to combat breast cancer.

A distinguishing feature of this article is that the key attributes of various preclinical breast cancer models and their utility are developed from the perspective of noninvasive molecular imaging. Despite major successes and lessons learned from the genomic landscape of cancer, it is now widely recognized that individual cancer genomes, like individual patients, are exquisitely heterogeneous; each contains a unique spectrum of drivers accompanied by passengers of less obvious significance. Tools that illuminate the cellular and molecular underpinnings of tumors on a patient-by-patient basis, such as noninvasive molecular imaging, will be essential to bringing precision cancer therapy to fruition. As such, preclinical imaging techniques relevant to mouse models of breast cancer, with an emphasis on molecular imaging, are also discussed in some detail.

MICE AS MODELS OF HUMAN CANCER

Although it might seem obvious, it is worth noting at the outset that all "models" of human disease are imperfect. Regardless of the degree of sophistication, model systems are, by definition, not humans. Rationales for late-phase clinical failures of new drugs are frequently based on a (healthy) skepticism of the translational value of certain preclinical models; much has been written about this issue already, and the value of model systems as a translational bridge to clinical applications is not debated in this article. However, in vivo modeling provides gains to the breast cancer field that complement what can be discovered at the laboratory bench. Indeed, the strongest experimental approaches will test hypotheses in multiple model systems. Therefore, it is critical to understand both the strengths and the limitations of in vivo models of breast cancer to maximize what can be learned with this approach.

The laboratory mouse (*Mus musculus*) represents a truly ideal model system for simulating the entire spectrum of events that lead to advanced breast cancer in humans. Mouse model systems enable elucidation of distinct facets of cancer biology that may not be frankly addressable in patients. Some of the advantages of the mouse as a model system are as follows: it is a mammal of small size, facilitating inexpensive housing and convenient handling; rapid breeding can facilitate colony expansion on convenient time scales; it has a relatively long life-span (~3 y); the complete sequence and characterization of the mouse genome are available; and manipulation of the mouse genome can be accomplished with relative ease. Additionally, mice and other rodents share many physiologic similarities with humans (*8*) and therefore are commonly used in drug metabolism and pharmacokinetic and toxicity studies. Ironically, for imaging studies, the small size of the mouse can be a limitation, particularly when studies aim to image tumors whose diameters approximate or are smaller than the effective resolution of the imaging modality of choice. Some notable differences between humans and mice include a higher metabolic rate in mice, an altered telomere length in inbred mouse strains, and an altered time frame for cancer onset (*9*).

HUMAN BREAST CANCER SUBTYPES: WHAT MODELS AIM TO RECAPITULATE

Several clinical and pathologic features of human breast cancer that allow stratification of patients on the basis of risk, prognosis, and likelihood of a response to certain types of therapy have been identified (*10*); in this light, for clinical breast cancer there are several impressive precision medicine–related success stories (*11*) and opportunities for future drug development (Table 1). Distinct molecular subtypes can be initially stratified on the basis of hormone receptor status; luminal breast cancers are typically hormone receptor–positive, whereas human epidermal growth factor receptor 2 (HER2) and basallike breast cancers (BLBCs) are hormone receptor–negative. Other potential molecular subtypes, including luminal C and normallike tumors, have been reported; at present, however, little is known about these subtypes (*10*).

Luminal A and Luminal B Subtypes

Luminal breast cancers are characterized by the expression of the estrogen receptor (ER) and the progesterone receptor (PR), which are nuclear hormone receptors, and other associated genes (*12*). Taken together, luminal A and luminal B subtypes account for approximately 65% of all breast cancers, although there are some differences between these subtypes. Luminal A breast cancers tend to express greater quantities of hormone receptors, particularly the PR, than luminal B breast cancers. In contrast, luminal B tumors tend to exhibit characteristics associated with higher-grade disease, are frequently more proliferative, are clinically more aggressive, and have a poorer prognosis than luminal A tumors. Because of their hormone receptor expression and activity, luminal A and luminal B breast cancers are routinely treated with endocrine therapies that block ER activity, including selective ER modulators (such as tamoxifen), selective ER downregulators (such as fulvestrant), and aromatase inhibitors (such as letrozole) that block the systemic production of the native ligand (β-estradiol). Luminal A and luminal B tumors exhibit disparate responses to chemotherapy, with higher-grade luminal B tumors frequently responding more favorably to chemotherapy (*10*). Given the hormone receptor expression and activity of luminal A and luminal B breast cancers, PET imaging with an ^{18}F-labeled form of estradiol (16α-^{18}F-fluoro-17β-estradiol [^{18}F-FES]) is often useful and may represent a suitable companion diagnostic approach for predicting a response to anti-ER therapy in selected patients (*13,14*).

HER2-Enriched Subtype

The *HER2* gene is amplified in approximately 15% of invasive breast cancers. Some breast cancers of this subtype have been shown to express ER, but most HER2-enriched tumors lack ER or PR expression. HER2-enriched tumors are frequently higher-grade tumors, with positive lymph node involvement. Precision medicine approaches to this cancer include the use of trastuzumab (Tz) (Herceptin; Genentech), a monoclonal antibody that selectively targets the *HER2* gene product, a receptor tyrosine kinase, as well as small-molecule kinase inhibitors (lapatinib and everolimus) (*15,16*). HER2-enriched breast cancers with metastatic disease are additionally treated with anthracyclines (doxorubicin) and often display an initial response to treatment, although recurrence is seen in nearly all cases. Other strategies targeting the HER2 receptor and its pathway include novel small-molecule inhibitors and HER2 antibodies, heat shock protein 90 inhibitors, agents targeting downstream components of the HER2 signaling pathway, and antibody–drug conjugates. Certain molecular imaging strategies targeting HER2-enriched tumors have leveraged the selectivity of Tz labeled with a positron-emitting isotope (^{64}Cu or ^{89}Zr). Promising clinical results in patients with metastatic breast cancer have been shown for these strategies (*17,18*).

BLBCs

BLBCs abundantly express epithelial genes, such as those for cytokeratins 5 and 17, but the expression of ER, PR, and HER2 is notoriously absent. On the basis of their lack of ER, PR, and HER2 expression, many BLBCs are deemed "triple-negative breast cancer" (TNBC). BLBCs are especially common in African American women (*10*) and are generally associated with a poor prognosis. Given the typical lack of ER, PR, and HER2 expression in BLBCs, molecularly targeted agents used to treat other breast cancer subtypes are often highly ineffective for BLBCs; therefore, chemotherapy is a mainstay for treating BLBCs (*19*). However, recent efforts to develop increasingly effective therapies against TNBC have led to the identification of several novel TNBC subtypes distinguishable by gene expression profiles and with potential vulnerabilities (*20*). Provocatively, noninvasive imaging of the androgen receptor by PET with 16β-^{18}F-fluoro-5α-dihydrotestosterone (^{18}F-FDHT), a structural analog of 5α-dihydrotestosterone, may represent a companion diagnostic approach for this challenging subtype. At present, a study is exploring the feasibility of using ^{18}F-FDHT PET to assess androgen receptor expression in metastatic breast cancer (ClinicalTrials.gov NCT01988324); this study is examining whether the effects of antiandrogens on tumor ^{18}F-FDHT uptake could aid in identifying optimum dosing for blocking the androgen receptor in metastatic breast cancer.

ATTRIBUTES OF PRECLINICAL MOUSE MODELS OF CANCER

Rapidly increasing knowledge about breast cancer molecular subtypes may affect the genesis of, progression of, and therapeutic strategy for any given breast cancer and underscores the importance of mouse model selection in designing preclinical studies and coclinical trials. Astounding growth in the reported number as well as the biologic elegance of mouse models for cancer research has been witnessed in the last decade. An extensive repertoire of mouse models with which to study breast cancer progression and treatment is now available. In genetically engineered mouse

TABLE 1
Major Subtypes of Human Breast Cancer

Molecular subtype	Gene expression features	Clinical features	Treatment and prognosis
Luminal	Elevated expression of hormone receptors and associated genes (luminal A > luminal B)	~65% of invasive breast cancers are ER- or PR-positive	Respond to endocrine therapy (responses to tamoxifen and aromatase inhibitors may differ in luminal A and B cancers)
		Luminal B cancers tend to be of higher histologic grade than luminal A cancers	Variable response to chemotherapy (greater in luminal B cancers than in luminal A cancers)
		Some overexpress HER2 (luminal B)	Prognosis is better for luminal A cancers than for luminal B cancers
HER2	Elevated expression of HER2 and other genes in amplicon	~15% of invasive breast cancers are ER- or PR-negative	Respond to trastuzumab (Herceptin)
			Respond to anthracycline-based chemotherapy
	Low expression of ER, PR, and associated genes	High probability of being high-grade and node-positive	Prognosis is typically poor
Basallike	Elevated expression of basal epithelial genes and basal cytokeratins	~15% of invasive breast cancers	No response to endocrine therapy or trastuzumab (Herceptin)
	Low expression of ER, PR, and associated genes	Most are ER-, PR-, and HER2-negative (TNBC)	Appear to be sensitive to platinum-based chemotherapy and polyadenosine ribose polymerase inhibitors
	Low expression of HER2	*BRCA1* dysfunction (germ line, sporadic)	Prognosis is typically poor (but not uniformly poor)
		Particularly common in African American women	

Adapted with permission of (*10*).

models (GEMMs), the tumor develops through all stages of epithelial transformation with the native stroma, immune system, and microenvironment (*21*). This trend has been propelled in part by the sheer volume of laboratories developing and deploying innovative mouse models to advance basic cancer research as well as by the adoption of contemporary and comparatively inexpensive genome editing technologies, such as the clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system (*22*) and RNA interference approaches (*23*). Another important contribution to the volume of mouse models recently described has come from the assembly of patient-derived xenograft (PDX) banks and, particularly for some cancer types, standardization of the infrastructure and protocols required to support these systems (*24*). Here we describe 4 types of mouse model systems that can be used for breast cancer research, identifying both the strengths and the limitations of each (Table 2).

Cell Line Xenograft Models

Mouse models of breast cancer derived by transplanting immortalized human cancer cell lines into an immunocompromised murine host are among the simplest and most frequently deployed model systems in cancer research. Most preclinical drug treatment studies performed in vivo have involved the use of immortalized human breast cancer cell lines growing within the subcutaneous dorsal flank of immunocompromised mice. Given the vast research history accumulated for many immortalized breast cancer cell lines and the numerous, diverse cell lines that represent all breast cancer molecular subtypes, xenografting breast cancer cell lines has become a staple in preclinical breast cancer research.

Although these models are technically simple to establish and are inexpensive to maintain over the short term, they have critical weaknesses that should be considered before larger programmatic efforts are based solely on them. In particular, shortcomings inherent in cell line xenograft models are commonly cited as the Achilles' heel of drug discovery efforts, especially when preclinical and clinical results are incongruent (*25*). An insightful commentary suggested that cell line xenografts are useful as a bridge between in vitro and in vivo studies (*3*). Objectively, cell line xenograft models have clear strengths, especially for rapid hypothesis testing, including the following: the development and extensive characterization of a panoply of human breast cancer cell lines from all molecular subtypes; the development of tumor stromal characteristics that can mimic the characteristics of human tumors (albeit incompletely); easily interrogated tumors; and quick tumor manifestation, which

TABLE 2
Preclinical Murine Models of Human Cancer

Model	Main components	Advantages	Limitations	Time and Cost*
Xenograft (cell line)	Immortalized human tumor cell lines transplanted into immunodeficient host (mouse)	Numerous established and well-annotated cell lines	Immunodeficient host	2–4 wk, $
		Representation from various human tumor types	Subcutaneous location may not allow cultivation of key tissue-specific stromal infiltrate	
		Features of tumor microenvironment, including stromal and vascular cells, incorporated within tumor	Cross-species divide; stromal components are mouse, whereas tumor cells are human	
		Tumors are easily and precisely measured	Limited or no genetic heterogeneity present within tumor	
Xenograft (patient-derived)	Human tumor explant propagated in immunodeficient host (mouse)	Heterogeneity and genetic diversity within tumors	Immunodeficient host	8–24 wk†, $$$
		Representation from various human tumor types	Subcutaneous location may not allow cultivation of key tissue-specific stromal infiltrate	
		Features of tumor microenvironment, including stromal and vascular cells, incorporated within tumor	Surgical implantation required	
		Tumors are easily and precisely measured	Cross-species divide; stromal components are mouse, whereas tumor cells are human	
			Genetic and phenotypic drift with passage	
Syngeneic	Immortalized mouse tumor cell line allografted into immunocompetent host (mouse)	Presence of intact immune system	Limited number of established cell lines, which are poorly annotated	2–4 wk, $
		Features of tumor microenvironment, including stromal and vascular cells, incorporated within tumor	Strong immunogenicity of some lines promotes spontaneous regression	
		All cell types within tumor are of mouse origin	Rapid growth rate of many lines limits use in longer-term studies	
		Tumors are easily and precisely measured		
GEMMs	Genetic modification that permits induced or spontaneous tumor development	Tumors develop in tissue of origin	Limited genetic mosaicism and heterogeneity of tumors	12–24 wk†, $$
		Presence of intact immune system	Technical hurdles for monitoring tumor response in internal organs	
		All cell types within tumor are of mouse origin	Low throughput and high investment	
		Features of tumor microenvironment, including stromal and vascular cells, and immune system components		

*$=low cost; $$=intermediate cost; $$$=high cost.
†Up to 1 y to observe metastases.
Adapted with permission of (*21*).

reduces attendant housing costs and speeds discovery. These strengths are balanced by the following limitations of cell line xenografts: the immunodeficiency of the host in which tumors arise, resulting in major contributions from the immune system to cancer development, cancer progression, and a therapeutic response being ignored; subcutaneous tumor propagation, which fails to simulate organotypic tumor microenvironments; a species disconnect between the tumor cells (human) and the stroma (mouse); and extreme homogeneity within the tumor, which poorly reflects the intratumoral heterogeneity seen in clinical breast tumors.

PDX Models

An often overlooked shortcoming of the cell line xenograft model is the fact that immortalized cell lines are developed through clonal attrition, resulting in cell populations that are propagated through multiple passages on a (typically) plastic surface. Selective pressures and genetic drift give rise to genotypic and phenotypic changes that may irreversibly distinguish daughter clones from paternal tumors (*26*); this scenario may poorly recapitulate the original underlying cancer biology of the patient. Models developed from patient-derived tumors, otherwise known as PDX models—in which patient tumors are surgically implanted into recipient murine hosts without being cultured—overcome this limitation. PDX models of various human tumors have been developed with great success, although breast cancer PDX models have historically been especially challenging (*27*). DeRose et al. reported exemplary success when multiple PDX tumor models derived from patient specimens recapitulated ER- or PR-positive, ER- or PR-negative, and HER2-positive tumors and TNBC (*28*).

The major strengths of the PDX approach include genetic diversity and heterogeneity that more accurately reflect human breast cancer; the ability to model various cancer subtypes; the incorporation of contextually correct human stroma within the tumor, including vascularity and inflammation; the documented ability to model metastasis; and easy interrogation of tumors, such as breast cancers, for correlative studies. This approach maintains the genetic and phenotypic integrity of the tumor cells, without the clonal selection or inadvertent genetic drift seen in immortalized breast cancer cell lines. PDX models are increasingly being used on the basis of the observation that the histologic and molecular (gene expression and copy number variations) characteristics of the PDX can be maintained through several mouse "passages." Importantly, PDX models retain clinical responses to many drug treatments, making them ideal for coclinical trials.

Nevertheless, there are several potential drawbacks of PDX models, including the requirement to use a severely immunodeficient murine host; the fact that the surgical procedure for implanting tumors into mice is invasive and requires skill (*29*); a species disconnect between the implanted tumor cells and stroma (human) and subsequently infiltrating stroma (mouse); and the time required to generate the models, which can require several months simply for the establishment of engraftment (*30*). Technical issues aside, the fact that establishing and maintaining PDX model systems require major capital investments in supporting infrastructure and personnel must not be overlooked.

Syngeneic Models

The requirement for the use of immunocompromised mice in xenograft models fails to incorporate the impact of the immune system on the tumor response. This area of cancer research is in its early stages, with rapid progress and vast promise that underscores the need for immunocompetent models of breast cancer for more rigorous analyses. Adequately modeling cancer immunology requires a propagating tumor within an immunocompetent host. One approach is to use mouse mammary tumors or mouse mammary tumor cell lines implanted into syngeneic immunocompetent murine hosts. Devoid of the species constraints inherent in xenografts and xenotransplants, allografted mouse tumors are not typically rejected by the murine host, given the similar genetic backgrounds. Syngeneic model systems offer the distinct advantage of studying cancer biology within the context of an intact immune system and species-specific tumor microenvironment. However, mouse tumor cell lines are limited and annotated to various degrees, and although small-molecule therapies may be adequately evaluated within these models, the species specificity of antibody imaging agents and therapies generally precludes their evaluation in syngeneic model systems.

GEMMs

GEMMs are the most sophisticated in vivo platforms used to simulate human cancer. These models are capable of not only accurately mimicking many relevant pathophysiologic features of human cancer but also recapitulating the sequence of molecular events that give rise to cancer. The transgenic expression of an oncogene specifically within the mouse mammary epithelium under the control of a strong mammary epithelial promoter is frequently used to induce mammary tumor formation. This is a clinically relevant model of tumor initiation and progression, enforcing the stepwise procession of cells from hyperplasia to ductal carcinoma in situ and then to invasive ductal carcinoma. Importantly, this process occurs within the context of the native stromal matrix (requiring stromal remodeling and angiogenesis) and the native immune system (requiring immune system evasion). The genetic manipulations can drive oncogene expression in a reversible or irreversible manner, in a tissue-specific manner (*3*) or, more broadly, throughout an entire organism. Frequently, GEMMs that harbor oncogenic driver genes (e.g., *HER2*) or lack tumor suppressor genes (e.g., *p53*), thus genetically mimicking human cancers, are developed.

The diverse array of oncogenes used to generate transgenic models of breast cancer has resulted in a multitude of models that mimic many of the specific molecular subtypes seen in clinical breast cancers, as confirmed by comparative expression analyses of mouse and human breast tumor samples (*31*). The advantages of GEMMs include tumor formation in the contextually appropriate tissue and potentially cell of origin through the use of tissue-specific or cell-specific promoters; an intact immune system; and a native tumor microenvironment that more accurately reflects human disease, including stromal components, vascularity, and inflammation. However, GEMMs are limited by the time, expense, and resources required to derive, establish, and maintain them; these demands can be overly burdensome given the potentially low experimental throughput of GEMMs. Few GEMMs of breast cancer truly harbor ER expression, despite commonalities in expression profiles between mouse and human luminal breast cancers. Although metastases in mouse breast cancer models are hematogenous and almost exclusively pulmonary, human breast cancer metastases occur though lymphatic spread that often precedes hematogenous metastasis to the lungs, liver, bone, brain, and elsewhere.

Molecular Imaging Applications: Biomarkers, Drug Discovery, and Coclinical Trials

Molecular imaging is an indispensable tool uniquely poised to address major challenges obstructing the delivery of personalized cancer therapy. Capable of noninvasively quantifying the cellular

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