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Animal models for exploring the pharmacokinetics of breast cancer therapies

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Abstract

Introduction—Despite massive expenditures in research and development to cure breast cancer, few agents that pass preclinical trials demonstrate efficacy in humans. Although this endeavor relies on murine models to screen for efficacy before progressing to clinical trials, historically there has been little focus on the validation of these models, even in the era of targeted therapy where understanding the genetic signatures of tumors under study is critical.

Areas covered—This review includes the transgenic, xenograft, and syngeneic murine breast cancer models, the ectopic, orthotopic and intravenous methods of cell implantation, and the ethics of animal experimentation. It also includes the latest data on tumor gene expression and the issues to consider when exploring the pharmacokinetics and efficacy of breast cancer therapies.

Expert opinion—Breast cancer drug development is expensive and inefficient without a consensus preclinical murine model. Investigators must approach the choice of murine model with the same sophistication that is applied to the choice of *in vitro* assays to improve efficiency. Understanding the limitations of each model available, including the nuances of tumor gene signatures, is critical for investigators exploring the pharmacokinetics and efficacy of breast cancer therapies, especially in the context of gene profiling and individualized targeted therapy.

Keywords

breast cancer drug development; murine *metastatic* model

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1. Introduction

Breast cancer is the second leading cause of cancer death in women in the United States [1]. On average, women have a one in seven chance of developing breast cancer and a one in thirty-five chance of dying from it [1]. Accordingly, society invests vast amounts of human, economic, institutional and intellectual capital in search of a cure [2]. Although billions of U.S. dollars have been invested in understanding and curing breast cancer, large portions of these investments have been lost in efforts which have failed to deliver. In fact, for the development of just one breast cancer drug, on average \$610 million and 37 months in animal trials are expended [3]. The large cost of bringing novel therapeutics from the tube on the bench to the patient in the clinic is partly due to the complexity of how to translate cancer cells in the petri dish to the human disease. One mechanism of bridging the wide chasm between basic scientists in the lab and clinicians in the clinic is testing novel cancer therapies in animal systems that model human disease. In fact, researchers use animal models as screening mechanisms to predict what new therapies show enough promise to warrant the investments required for human clinical trials. However, despite these screening efforts, a cure has not been discovered and many therapeutics that showed promise in animals failed to deliver in humans [4-9]. The challenge is to utilize breast cancer drug development models that more efficiently induce cancer progression, produce the least confounding variables in interpreting results, and provide endpoints that are clinically relevant to human breast cancer [4,8,10-14]. In fact, a recent editorial by Dr. Breyer in this journal called for critical evaluation of these models that includes such clinical endpoints as well as gene signatures which are of increasing salience in the era of targeted therapy [15].

Over the last 20 years, there has been an exponential growth in the number of publications in the literature on breast cancer, an increase in the number of different breast cancer metastasis models in use, and our understanding of breast cancer biology has become increasingly complex. In fact, breast cancer research has advanced to focus on the genetic signatures of tumors, which predict cancer biology and provide candidate targets for individualized chemotherapeutic interventions[16]. The promise of this new knowledge to influence the prognosis and treatment of breast cancer is such that even the 2010 edition of the TNM cancer staging manual predicts its impact on future editions [17]. However, despite the impressive advances in breast tumor genetic profiling and despite a major shift toward targeted chemotherapy which focuses on these specific differences among tumors, the science of metastatic breast cancer animal modeling has failed to keep up. The result has been that this critical translation bridge has failed to meet the challenge. Although increasing resources have been dedicated to developing increasingly sophisticated interventions, it has been unclear how well these animal models serve the purpose of adequately predicting the promise of curing breast cancer in humans. Until recently investigators have not integrated tumor gene profiling into a critical evaluation of breast cancer metastatic animal models [4,5,7,8,11-14,18,19].

Modeling human breast cancer in mice for drug development has historically been thought of in two thematic ways. First, the focus has been to study this disease in a mouse system that mimics human cancer progression [4,8,10-14]. Second, the approach has been to determine the efficacy of novel therapeutics in mice with endpoints that are clinically

significant to human cancer [4,5,7,8,11-14]. Applying these two main priorities, the literature has evolved multiple methods of modeling metastatic breast cancer: transgenic mice, implantation of human xenografts into immune-deficient mice, implantation of mouse derived breast cancer into immune competent syngeneic mice, orthotopic implantation, ectopic subcutaneous implantation, and intravenous implantation methods. The purpose of this review is to discuss the benefits and limitations of each method, with a focus on the advances made in breast cancer biology and targeted chemotherapy. Furthermore, the objective is to assist investigators in choosing the appropriate model that will improve the efficiency of the breast cancer drug development process.

2. Preliminary factors to consider

As with the use of any assay, the first factor which researchers must consider is what scientific question they seek to answer. Using the scientific aim as a litmus test for whether a proposed animal model system for breast cancer is appropriate will maintain the focus of the project to produce data that are meaningful to the hypothesis in question. Therefore, a critical examination of the available models should be performed, with a keen focus on the scientific objective, before proceeding with *in vivo* experimentation. Unfortunately, however, the experience and knowledge of the researcher, rather than the translatability of the mouse system, often predict which cell lines and which mouse species are used [4,5]. Too often, then, novel therapeutics progress from such studies without being critically evaluated to determine bias, efficacy, and how well the model system translates to human cancer biology [4-9].

At the outset, researchers must determine whether to implant human breast cancer into immune-deficient mice or mouse breast cancer into immune-intact mice [4,6,7,14,20]. While the costs and benefits of xenograft versus syngeneic models will be discussed at length below, at this point it is important to consider how these models generally pertain to the hypothesis being tested. Namely, the researchers should consider what type of immune response and to what extent it plays a role in testing their hypothesis and the extent to which ignoring the role of immune functions may produce confounding results. Along the same line of reasoning, the researchers should also consider the extent to which tumor-microenvironment, tumor-stromal, tumor-epithelial, and tumor-host interactions play a role in testing the hypothesis and/or in producing confounding results. Answering these questions is critical because the use of immune deficient mice ignores cancer immunology, obliterating the mammary fat pad architectural contiguity with host tissues potentially confounds cancer-host tissue interactions, and the use of alien human tumor tissue implanted to the foreign tissue of mice confounds efforts to test these interactions [4-7,9,14,20-24]. Once these questions are answered, the researchers must consider what endpoints must be evaluated in the generated data to accomplish their scientific objectives, and therefore to what extent each model can produce data that allow for measuring those endpoints.

In accomplishing the goal of bridging the chasm between the bench and the clinic, the ideal animal model best mimics breast cancer metastasis and provides endpoints that are of significance in human breast cancer [4,5,7,8,11-14]. The first challenge for many researchers is determining what models meet these twin objectives for the purposes of

testing their hypothesis. Strategically, it is useful to collaborate with clinicians and veterinarians in order to evaluate to what extent the animal model progresses in a manner analogous to human breast cancer progression, and the extent to which the endpoints correspond to those of significance in humans, such as disease free and overall survival in humans. First, to what extent does the animal model reproduce the biological steps of cancer progression and metastasis? To what extent does this compare to the natural history of breast cancer in humans? And to what extent does this apply to the hypothesis being tested? Second, what kind of data are going to be generated by the model vis-a-vis clinically relevant variables? For example, does the model only allow for surface area measurements of local tumor growth, or are there dynamic modalities available to monitor cancer progression and metastasis reliably and minimally invasively? To what extent will a positive result be of pertinence in the context of how human breast cancer is understood and/or treated in humans? These questions are critical in terms of not only assessing the degree to which each model may or may not be appropriate for attesting the hypothesis in question, but these questions also serve to focus the researchers on whether the hypothesis itself is aimed at answering a translatable breast cancer research question in the first place. Furthermore, it will save time and resources, as well as facilitate establishing efficient collaborations with clinicians and veterinarians to maximize the translatability of the results. Finally, when the appropriate mouse model is identified, it is important to validate the degree to which it reliably produces the data and endpoints desired before evaluating the efficacy of any novel therapeutics or testing novel hypotheses of breast cancer biology *in vivo*.

3. Transgenic mouse models

As is commonly known, humans spontaneously develop breast cancer and a great deal of research and development has been invested in understanding tumorigenesis and preventing its occurrence. However, mice do not spontaneously develop breast cancer [14]. Instead, researchers have focused on genes that are known to promote the formation of cancer as well as genes that suppress the formation of cancer, in addition to other genes, as a means of understanding how breast cancer develops in humans. In addition, by genetically altering mice to over-express tumorigenic genes or to under-express tumorsuppressive genes, models have been developed where mice spontaneously form breast tumors.

One of the most commonly used models are the polyoma virus middle T oncoprotein (PyMT) and HER2 overexpression transgenic mouse models. PyMT, which is not expressed in human breast cancer cells, utilizes the mouse mammary tumor virus LTR to over-express the membrane-attached protein on mouse mammary epithelium [25]. The result is mammary hyperplasia in 4 weeks and carcinoma in about 14 weeks and the progression of metastasis is from the primary tumor to the regional lymph node, followed by the lungs[25]. However, the expression profile of estrogen, progesterone, erbB2/Neu, cyclin D1, and integrin- β 1 varies in the same tumors of the mice over the course of their disease, which does not reflect the standard progression of human breast cancer [25]. In fact, DNA microarray analysis comparing PyMT produced tumors to human samples was limited by estrogen receptor and estrogen receptor-regulated genes within these murine tumors [26]. While this model provides an interesting system to explore the progression from mammary hyperplasia to late

carcinoma, it is important to understand these limitations for exploring the efficacy of novel therapeutics.

Kunming mice are the largest number of closed colony mice in production in China which spontaneously form estrogen receptor negative, progesterone receptor negative, and HER2 expressing mammary tumors [27]. These mice have been shown to produce tumors that are weakly expressing the HER2 protein by immunohistochemistry, but have a high vascular endothelial growth factor, c-Myc and cyclin D1 [27]. While these tumors do metastasize to distant organs such as the liver and lung, they do so hematogenously rather than via the lymphatic system, which is in stark contrast to human breast cancer progression [27]. There have been efforts to utilize such spontaneous models as well as transgenic HER2 models to test novel therapeutics for chemoprevention in breast cancer; however, the authors concede that the dramatic differences between these models and human breast cancer greatly limit the applicability of their results outside of clinical trials [28]. The hypothetical benefit of these models is that they potentially provide an *in vivo* system testing hypotheses on how to prevent breast cancer. However, there are several limitations to this approach.

First, the tumors that these mutated mice form in the mammary fat pad usually exhibit limited local invasion and rarely metastasize [14]. Even when they do invade and metastasize, these mice form so many tumors in different sites, it is a challenge to monitor the impact of interventions on these multifocal lesions. These models therefore pose a challenge for researchers seeking to investigate the prevention of aggressive breast cancer. Furthermore, for researchers seeking to translate hypothetical molecular mechanisms of cancer progression and metastasis, or testing interventions targeting those mechanisms, such models will produce findings that are not linked to any relevant endpoints because those specifically targeted mechanisms are absent. Second, estrogen, progesterone and Her2/neu receptor status play an important role in the prognosis and treatment of human breast cancer. However, it should be noted that the majority of the tumors these transgenic mice form lose their estrogen receptivity, and therefore are limited in their suitability for studying the role of these clinically significant receptors [14]. Third, the targeted alterations of one, or even a few genes produce potentially confounding variables, or at least oversimplify the exceptionally complex, multi-allelic, and multi-factorial process of human breast cancer tumorigenesis and prevention. Finally, it usually takes months until these transgenic mice develop breast tumors, which make this model not practical as a drug development tool from a cost and efficiency stand point. Although transgenic mouse models have the benefit of spontaneously generating tumors without surgical manipulation, thus potentially analogous to human tumor from a tumor development point of view, their functional utility as a translational tool is limited.

4. Xenograft mouse models

When considering the appropriate *in vivo* system for translating *in vitro*-based hypotheses to the clinics, the idea of using human breast cancer tissue in a mouse seems appealing at first glance. Mouse models of patient tumors are more related to the patient than high-passage cancer cell lines, and therefore provide an attractive system for translational research [8,29]. However, these models should be approached with a great deal of caution, guided by

a disciplined critical attention to what hypothesis is to be tested. In the first place, the human breast cancer in the context of a mouse is quite different than the same cancer in the context of the human being in which it arose. As stated above, it is critical to consider at the outset to what degree immune response can affect the variables testing the hypothesis because immune deficient mice must be used in order to minimize the rate at which the mice reject the human tissue. For example, this inflammatory rejection is not completely eliminated in nude mice because it has been demonstrated that subcutaneous implantation can still trigger the host to react to the growing tumor and form a scar-like capsule around it, reducing the tumor size compared to orthotopically implanted cells [19].

In addition, researchers must consider the degree to which cancer-host interactions can affect the variables testing the hypothesis because these interactions are potentially confounded by having human derived cancer interacting with mouse host tissue. Furthermore, it should be noted that these samples usually demonstrate an indolent course of progression and often only develop metastases several weeks to months after implantation, while many xenograft samples do not metastasize at all [4]. Patient-derived orthotopic xenograft (PDOX) mouse models replicate the natural course of the patient tumor, including metastasis [30-36]. In contrast, subcutaneous transplant models of patient tumors in nude mice, which now have names such as “tumorgraft” [37] or “xenopatient” [29], or “avatar” [38], do not metastasize.

In the situation of testing novel therapeutics, any results stemming from such models should be viewed with caution because of the importance of these altered factors in human breast cancer biology and treatment. In a sense, xenograft models are the Trojan horse of mouse breast cancer modeling, by offering the promise of a humanized animal system, but instead only introduce multiple alien factors that further confound and artificialize the *in vivo* system. However, if a xenograft model is considered appropriate for testing the hypothesis, then there are additional factors to consider.

First, it is important to consider factors unique to the use and handling of human tissue. As a measure of caution, there are specific regulatory safeguards which must be taken to protect the identity of the patient donors of these samples. It should be noted that there is a great deal of heterogeneity between human tumor samples, even in the best of scenarios. In addition, given the communicability risk that these human breast cancers pose to investigators performing experiments and handling the animals, additional regulations require strict compliance to protect all individuals involved. Third, it is important to evaluate the degree to which the tissue provided represents the purported databank tissue characteristics identified, the maximum number of cell passages possible before the fidelity of the cancer to its original character is lost, and the appropriate quality control measures required to maximize the viability of the human cancer cells. Unfortunately, due to these quality control issues human xenografts often produce variable uptake in mice, variable phenotypes and progression patterns between mice, and they consequently often produce false positive results [4]. Finally, it is important to consider utilizing other animal models to confirm the findings produced by this model in order to address the shortcomings of this *in vivo* system.

5. Syngeneic mouse models

The benefit of implanting mouse derived breast cancer cells into mice of the same genetic background is two-fold. First, this model utilizes immune competent mice which allows for evaluating the role of immune response in breast cancer progression and metastasis [4-7,9,14,20-24]. Second, because the cancer cells are of the same genetic background as the mice, their interaction with host tissue will not be confounded by inter-species interactions. In fact, there is evidence that the 4T1 mouse derived breast cancer cell line implanted into immune-intact syngeneic mice mimic human cancer progression and metastasize more efficiently than human xenografts implanted in immune-deficient mice [21-24]. Given the recent realization of the importance of tumor-promoting inflammation in cancer progression[39], having the immune-response component intact in the model to test drug efficacy can be critical. Namely, these tumors not only produce larger tumors faster than xenografts, but they also metastasize more rapidly and consistently, thus reaching clinically relevant endpoints more efficiently than xenografts. However, no model is without limitations.

Researchers using syngeneic mouse models must keep certain limitations in mind. First, as is most obvious, mouse breast cancer is different than the human disease. It is from the outset an artificial phenomenon created purely as a tool of cancer research, for mice do not form these cancers spontaneously [14]. However, it should be noted that mouse derived breast cancer cell lines, such as 4T1 cells from balb c or E0771 cells from C57/blk6 background, have been widely described and utilized in the literature so that the applicability of this cell line in its syngeneic mouse as a model can easily be determined and recognized by researchers in the field [22-24]. While these mouse derived cell lines are obviously not the same as human cancer, they are not plagued by the same quality control and heterogeneity shortcomings of human xenografts.

Second, as is perhaps as equally obvious, tumor bearing mice are not the same as humans with metastatic breast cancer. While it has been argued that at least with syngeneic models the immune response and cancer-host interactions are more faithfully preserved than in the xenograft system [4-7,9,14,20-24], it is important to keep in mind once again that there are important differences between the human host and the mouse host in cancer. The mouse immune system is considered to be more resilient than in humans, with important differences in innate and adaptive immunity [4]. Furthermore, there are known differences between human and mouse stroma in cancer, such as differences in mesenchymal stem cell malignant transformation [40].

Although there are important differences between humans and mice, at least this model maintains the homogeneity of breast cancer derived from a genetic background similar to the host animal. In addition, with a greater understanding of the importance of gene profiling in breast cancer [16], genetic profiling of tumors produced by these models can serve to guide researchers regarding which method is most appropriate for testing the hypothesis in question and for focusing on specific genes relevant for targeted therapy [18,19].

6. Orthotopic versus ectopic implantation

Once the appropriate cell line and mouse background have been selected, the next question which researchers must consider is the method of cancer implantation. Many have argued for orthotopic implantation directly into the mammary fat pad under direct vision (ODV) so that cancer cells can benefit from the microenvironment of the organ of origin [8,12-14,21,[41-44](#)], the majority use either ectopic subcutaneous injection (SQ) [4,7,10,11,14,[45,46](#)] or percutaneous injection blindly in the area of the nipple in attempt to implant the cells into the mammary fat pad (OP) [19]. Although advocates of ODV have argued that tumor microenvironment matters in drug development and have even cited multiple examples of SQ limiting the viability of cell lines, and despite evidence that ODV promotes progression and metastasis more efficiently than SQ [4,11-13,21,[42-44,46-53](#)], until recently research has not focused on critically examining these models [4,5,7,10-13,[33,36,52-68](#)]. In fact, over the last 20 years while the above evidence has been reported in favor of ODV, there has been a shift away from ODV towards SQ and OP [19], even though there is evidence that orthotopic models correlate with the bioavailability of therapeutics in the host's organ of origin [4,13] and that ectopic implantation in other cancers produced false positive results that were later contradicted by orthotopic implantation [[64,65,69](#)] and human clinical trials [7,[59,69-77](#)]. The arguments against ODV have been based on the perceived technical difficulties of ODV [4,10,[33,36,46,54,55,69,78](#)] and because it has not been definitively proven that ODV will discover drugs that SQ will not [4,10,13,[69](#)]. Until recently, there has been little evidence to critically address these concerns.

Although proponents of SQ have argued that it is easier to monitor cancer progression than ODV, the advent of noninvasive bioluminescent technologies has addressed this concern and have added the capability of objectively quantifying clinically relevant factors such as cancer progression, metastasis and overall tumor burden [[4,46](#)]. In addition, despite previously perceived conceptions of the technical challenges of performing ODV, this issue has recently been addressed [19]. Not only has it been recently shown that ODV is relatively easy to learn, even for researchers with minimal animal experience, it has also been demonstrated that it does not require that much more time to perform than SQ [19]. However, these issues are secondary when one considers the differences in the genetic profiles of tumors formed by SQ versus ODV.

The genetic profiles of tumors determine breast cancer biology and have also increasingly become the focus of developing targeted therapy against this disease [16]. It has been recently reported that there are significant differences in the gene expression of SQ and ODV tumors, differences in genes whose network of gene interactions have significance in cancer biology and therapy, and specifically in genes which are currently the focus of breast cancer research and targeted drug development [19]. The implications of these findings are that the implantation of breast cancer cells into the appropriate site is not merely an issue of limiting confounding factors in predicting response to therapy, ease of measuring the size of tumors or of injecting cancer cells in the hands of researchers. Instead the decision of whether to proceed with an ODV or SQ approach has direct consequences on the gene targets which researchers plan to investigate.

Considering the importance of implanting cancer cells into the mammary fat pad and making use of that organ of origin, it is important to consider how to effectively accomplish this objective. First, it has been demonstrated that the blind attempt of orthotopic implantation via OP is less accurate and more confounding than ODV [19]. Second, although the literature indiscriminately reports injecting either 10uL and 100uL volumes of cancer cells into the small mammary fat pads, it has also been demonstrated that less volume produces less mammary fat pad disrupt and consequently greater cancer progression than high volume despite the fact that less number of cells are delivered [19]. Third, although the literature indiscriminately reports ODV of the chest versus abdominal mammary fat pads, it has been demonstrated that chest ODV more efficiently promotes metastasis to distant organs than abdominal ODV [19]. Therefore, researchers who decide to pursue orthotopic implantation should proceed with the well described 10uL chest ODV method.

7. Intravenous injection models

Although breast cancer progresses from a primary tumor to metastasize to distant organs [8,14] and even though it has been reported that there is signaling between the primary tumor and metastatic lesions especially in breast cancer [79], many drug developers use SQ to evaluate a drug's effects on local progression by measuring tumor growth [8,80], and separately use intravenous injection methods (IV) with the intent to evaluate drug effects on metastatic progression by colonizing distant organs via cancer cell blood stream inoculation [8,14,79]. Advocates of the IV model argue that it is an easy and quick method to form metastatic lesions, especially in xenograft systems which take long periods of time to metastasize, if at all. However, there are several important limitations to consider.

First, researchers must examine to what extent testing the hypothesis requires a system that includes all the necessary biological steps which a primary tumor must take to produce a distant metastasis. If the answer to this question is in the affirmative, then it becomes problematic to utilize a model that has no primary tumor that must not undergo angiogenesis, lymphangiogenesis, invade, migrate, enter a lymphatic vessel, metastasize to lymph nodes and/or enter the blood stream, invade a distant organ and form a metastasis [8,14]. Instead, IV methods form distant organ cancer colonies after the blood stream is inoculated with cancer cells. In fact, the lesions formed and described widely in the literature constitute diffuse colonization of target organs, rather than discrete metastatic foci [18]. IV methods include tail vein injection to colonize the lungs, splenic and portal vein injection to colonize the liver, cardiac injection to colonize the bones, and carotid injection to colonize the brain. Although the genetic signatures of the metastatic lung tumors produced by IV versus those that metastasize from the primary tumor created by ODV to form discrete lung metastases, the IV method produced diffuse colonization of the lung with cancer that often produces mortality by thromboembolic phenomena, rather than by cancer progression [18]. This point is important to consider as a significant limitation of the IV method to produce relevant clinical endpoints for translational research.

Second, researchers must consider the extent of primary tumor - metastatic lesion interaction that is required to test their hypothesis. It has been reported that the primary tumor secretes angiostatin which inhibits the angiogenic proliferation of distant metastases [79]. This

phenomenon bears a great deal of relevance for breast cancer researchers because currently women with metastatic breast cancer do not routinely undergo mastectomy in order to prevent the removal of that inhibition of metastatic proliferation [81,82]. It should be noted that another critical component of this signaling includes the immune system [83], which is once again ignored in xenograft models. Therefore, testing novel therapies for women who will have both a primary tumor and metastatic lesions in a system that does not include that important primary tumor - metastatic lesion signaling dynamic may prove to be problematic in terms of translatability to the clinic.

8. Humane treatment of animals

The ethics of experimenting on animals are predicated on the principle that these investigations are undertaken in the pursuit of increasing scientific knowledge for the benefit of society and the individual suffering from breast cancer. The corollary to this principle is that all animal experimentation performed in achieving this noble goal be conducted in a way that minimizes the suffering of the animals [84]. In fact, in the United States Federal law mandates that institutions conducting animal research form self-regulating Institutional Animal Care and Use Committees which advance these objectives in collaboration with the American Association for Laboratory Animal Science (AALAS). Consequently, there are many rules and regulations established to further these principles and guide researchers on the humane use of laboratory animals.

The complexity of cancer biology and treatment, the inefficiencies of cancer drug development, and the challenges of translational mouse modeling of metastatic breast cancer should encourage researchers to collaborate with regulators on how best to proceed with experimentation. Because the interests at play are so multifaceted and the scientific and clinical issues so technically sub-specialized, it can seem challenging when researchers seek to navigate the regulatory world of animal research. However, researchers should be reassured that humane animal experimentation at its foundation requires that the best science be pursued in good faith. In fact, AALAS acknowledges the complexity of these issues and has consequently provided guidelines for researchers and IACUCs to follow so efforts intended to prevent suffering do not in any way limit the validity of the research[84]. These AALAS provisions are particularly important when considering sensitive *in vivo* assays that may be affected by exogenous narcotic administration, especially when considering how to measure clinically significant endpoints such as disease free and overall survival. Therefore, it is critical for researchers to deliberate with their IACUCs, in collaboration with veterinarians and clinicians, on how best to design protocols and morbidity criteria that protect the animals without preventing researchers from conducting strong science that bears the promise of discovering cures for women dying from breast cancer.

9. Conclusion

Without a consensus mouse breast cancer metastasis model in the literature, and with so much variability reported among the different models in use [4,7,10,11,14,45,46], it is no surprise that the breast cancer drug development process costs so much[3] and often produces conflicting results [7,59,64,65,69-77]. The purpose of this review has been to

guide researchers to approach the decision of which *in vivo* breast cancer system to use with the same discipline, sophistication, and critical evaluation that is applied to the choice of *in vitro* assays, so that there will be greater efficiency in the field of breast cancer research and drug development. Furthermore, the intent has been to do so in the context of the exciting era of breast cancer gene profiling and individualized targeted chemotherapeutic drug development. Therefore, applying these lessons in the context of the increasingly complex world of breast cancer will guide researchers to understand breast cancer and to develop new treatments at lower costs and with greater translatability to the clinic.

10. Expert Opinion

Breast cancer drug development is expensive and inefficient without a consensus preclinical murine model. Despite the massive expenditure of resources over decades by governmental and private institutions to cure breast cancer, historically little attention has been paid to the studies that validate how well murine models actually model human breast cancer in the first place. A better understanding of this issue is not just a matter of an efficient use of resources, it is critical issue for investigators determining whether their novel agent or pharmacokinetic pathway holds promise for a cure.

Investigators must approach the choice of murine model with the same sophistication that is applied to the choice of *in vitro* assays to improve efficiency. First, the investigator must consider the proposed mechanisms of action of their agent at the cellular and physiologic level. Second, the investigator should consider what steps along the path of metastatic progression their novel agent is hypothesized to act to produce a therapeutic effect. Finally, the investigator should then consider how each murine model evaluates or fails to evaluate these factors. If the degree to which the pathway in question is mimicked in murine breast cancer models is unknown, then the models should be validated first before proceeding with testing the new agent. This process must be considered before proceeding with preclinical trials and should also be considered when interpreting the results of those trials.

Understanding the limitations of each model available, including the nuances of tumor gene signature, is critical for investigators exploring the pharmacokinetics and efficacy of breast cancer therapies, especially in the context of gene profiling and individualized targeted therapy. Whether or not a novel agent is designed as a targeted therapy, investigators in breast cancer must consider its efficacy in the context of breast cancer subtype. At the very least, the cell lines used should be mapped to determine how well they compare to human breast cancer at the cellular level. Furthermore, genetic signatures of the tumors and metastases produced by the model under consideration should be evaluated to understand how well those models serve as a screen for the efficacy of a novel targeted therapy.

Breast cancer is a complex disease and the endeavor to understand and treat the disease accordingly has become increasingly complex. While there is no single preclinical model or biologic pathway that serves as an orthodox system to explore the pharmacokinetics of breast cancer therapies, there is a disciplined, systemic approach rooted in the scientific method for investigators to employ when considering preclinical trials. Applying this approach even in the context of the increasingly complex world of breast cancer will guide

researchers to understand breast cancer and develop new treatments at lower costs and with greater translatability to the clinic.

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Article Highlights

- Breast cancer drug development is expensive, inefficient and relies on preclinical animal models without a consensus model for investigating the pharmacokinetics of breast cancer therapies, which therefore requires investigators to understand the limitations of each model available.
- Investigators must understand the degree to which each mouse model tests the pharmacokinetic mechanism of their novel therapy and the gene expression of the tumors generated by that model.
- While the transgenic models theoretically provide a system to test genetic targets of pharmacoprevent and treatment for breast cancer, investigations are limited by the failure to either mimic human cancer progression or to produce tumors that are genetically stable for reliably testing the efficacy of a novel agent in vivo .
- While xenograft models theoretically provide a system to test efficacy on human tissue, the system is so artificial and confounded by multiple factors that any results from these models must be viewed with caution.
- Implantation of mouse derived breast cancer orthotopically into immune intact mice of the same genetic background provides the best model available; however, it is still important for investigators to understand the limitations of translating results in mice to applicability to humans.
- While ectopic implantation and tail vein injection are commonly used in the literature, recent reports highlight the limitations of these methods and demonstrate how misinterpretation of their results explain why many novel therapeutics that showed promise in the models failed in humans.