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Drug discovery in prostate cancer mouse models

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Abstract

Introduction—The mouse is an important, though imperfect, organism with which to model human disease and to discover and test novel drugs in a preclinical setting. Many experimental strategies have been used to discover new biological and molecular targets in the mouse, with the hopes of translating these discoveries into novel drugs to treat prostate cancer in humans. Modeling prostate cancer in the mouse, however, has been challenging, and often drugs that work in mice have failed in human trials.

Areas covered—Here we discuss similarities and differences between mice and men, types of mouse models that exist to model prostate cancer, practical questions one must ask when using a mouse as a model, and potential reasons that drugs do not often translate to humans. We will also discuss the current value in using mouse models for drug discovery to treat prostate cancer and what needs are still unmet in field.

Expert opinion—With proper planning and following practical guidelines by the researcher, the mouse is a powerful experimental tool. The field lacks genetically engineered metastatic models, and xenograft models do not allow for the study of the immune system during the metastatic process. There remain several important limitations to discovering and testing novel drugs in mice for eventual human use, but these can often be overcome. Overall, mouse modeling is an essential part of prostate cancer research and drug discovery. Emerging technologies and better and ever-increasing forms of communication are moving the field in a hopeful direction.

1. Introduction

Prostate cancer is the most diagnosed non-skin cancer in men in the United States, with approximately 230,000 new cases each year¹. The 5-year survival rate for patients with localized prostate cancer is 100%; however, the 5-year survival rate for patients with metastatic prostate cancer is about 30%. These numbers translate to approximately 30,000 prostate cancer-related deaths every year¹. It is now appreciated that patients may have indolent, intermediate, or aggressive disease, and it can be difficult to determine how to treat them. For patients with indolent disease, it may be harmful to perform interventions such as radical surgery, radiation, and chemotherapy. For those with aggressive disease, it is harmful to wait to treat with the same interventions because these cancers can progress relatively quickly. The prostate-specific antigen (PSA) blood test, combined with the digital rectal

exam, histology from biopsies, and imaging (e.g. bone scans) give clinicians information about cancers to assist in making treatment decisions. Unfortunately, patients are still being over-treated, and men are still dying from prostate cancer due to a lack of more detailed prognostic tests and effective drugs to treat the appropriate disease state. Metastatic prostate cancer, on the whole, remains incurable.

These gaps in knowledge require experimental models with which to advance knowledge and discover and test novel therapeutics. The mouse, *Mus musculus*, has been used extensively to model prostate cancer and to test novel drugs preclinically. The mouse is the single most important mammal with which we can conduct experiments to further our knowledge of cancer biology^{2, 3}. Mice are used in drug discovery to discover novel molecules involved in cancer, to determine whether cancer-associated molecules truly recapitulate the associated cancer property, and to test whether novel drugs are non-toxic and work to effectively combat cancer. The mouse has many advantages as a cancer model, and its use has led to many new discoveries. The mouse also has several disadvantages that may explain why so few drugs that work in mice do not work in humans. A lack of thorough understanding of how to use the mouse as a model may also hurt our collective ability to translate novel discoveries into novel drugs. In this review, we will discuss the advantages and disadvantages of using mice to model prostate cancer, the various types of models that have been used to do so, and we will end by providing our opinions as to the mouse's utility and the future of mouse models in prostate cancer.

2.1 Mice and men: how similar are the organisms?

How similar mice are to men is an important question. Men and mice have some obvious similarities, such as both being mammalian. There are also some obvious differences, in that the mouse is approximately 300 times smaller than the man, walks on all fours, and has a tail. There are many more subtle characteristics by which we can compare the two. The mouse genome (specifically the C57BL/6 strain) was sequenced in 2002 – one year after the human genome was completed. The human genome is approximately 3.3 gigabases (Gb) in size, whereas the mouse genome is 2.7 Gb. Approximately 40% of the human genome can be aligned to the mouse genome, and over 90% of the two genomic regions have conserved synteny⁴. About 99% of mouse genes have a human ortholog, and the nucleotide and amino acid sequences of these orthologs are approximately 85% identical^{3, 5}. There are of course differences between the sequences – telomere length (much longer in mouse)^{6, 7}; GC content (42% in mouse, 41% in human); and activity of transposable elements (more active in the mouse lineage), for example⁴.

Further genetic analysis was completed in 2014, when six seminal papers were published comparing the human and mouse genomes and transcriptomes, as well as regulatory mechanisms^{8–13}. It was found that while many genes share similar expression patterns, 4,767 genes were differentially expressed at a statistically significant level (2,569 were more highly expressed in humans, and 2,198 were more highly expressed in mice)¹². An example of differential expression is in hematopoietic stem cells (HSCs), which express the cell surface marker CD34 at a high level in humans, while expressing CD38 at a low level. Conversely, mouse HSCs express high levels of CD38 and low levels of CD34¹⁴. The data

also point to a high degree of divergence for sequences that are related to *cis*-transcriptional regulation and higher order chromatin organization¹¹. In any given experiment, genomic and gene expression differences must be taken into consideration when performing any experiments involving mice, and care and caution must be taken in designing any experiment.

2.2 Mice and men: how similar are the prostates?

There are noteworthy differences between mouse and human prostate expression signatures. While mouse prostate cells respond to androgen stimulation and signaling similarly to humans^{15, 16}, mice do not express certain human androgen-responsive genes, such as prostate-specific antigen (PSA) and prostate-specific membrane antigen (PSMA)^{17, 18}. More broadly, there are other genes that are differentially expressed but many that are similarly expressed between mice and human in normal prostate tissue¹⁹ and prostate cancer^{20–22}.

Mouse and human prostates differ anatomically. Human prostates are walnut-shaped and -sized, consisting of a single lobe and three zones (central, peripheral, and transitional) surrounding the urethra at the base of the bladder. The mouse prostate also surrounds the urethra at the base of the bladder, but is comprised of three pairs of lobes (anterior, ventral, and dorsolateral) one of which (anterior) protrude out immediately adjacent to the seminal vesicles (see Figure). Testosterone levels also differ – total and free plasma testosterone levels may vary between individual mice by 30-fold (likely due to episodic secretion²³) and vary greatly between genetic backgrounds²⁴. In addition, the average testosterone level of a hormonally intact mouse resembles hypogonadal human levels²⁵. Adding and removing testosterone from mice drastically affects prostate tumor growth in xenograft models²⁵.

Histologically, the mouse prostate is strikingly similar to the human prostate, and great care has been placed into defining and describing the characteristics of normal and neoplastic prostate histology²⁶. Mice differ in the ratio of basal cells to luminal cells, which is roughly 1:1 in humans but is 1 basal cell to 4 luminal cells in mice (see Figure)²⁷. Importantly, mice rarely, if ever, spontaneously get prostate cancer – in a study of 612 wild-type mice, none were found to have prostate carcinoma²⁸. This makes syngeneic models more difficult to generate and extremely rare, but the advantage is that the prostate cancer seen in mouse models is almost definitely caused by the experimental conditions, rather than by chance. Overall, the mouse prostate has many anatomic, histologic, and genetic similarities to the human and is a valuable mammalian organism with which to model human disease, as long as the differences are understood and taken into consideration.

2.3 Mouse models of prostate cancer

There are four main types of mouse models of prostate cancer: genetically engineered mouse models (GEMMs), cell line xenografts, patient-derived xenografts (PDXs), and syngeneic models. Each type has its own advantages and disadvantages (Table 1).

2.3a GEMMs

GEMMs can be broken down into three further categories: transgenics, whole-body knockouts, and conditional knockouts²⁹. Transgenics use tissue-specific promoters to cause

expression of a DNA construct. Whole body knockouts delete genes ubiquitously via homologous recombination in embryonic mouse stem cells³⁰. To avoid embryonic lethality and whole-body deletion, conditional knockouts were developed, in which genes are deleted in a tissue-specific manner, most often using the Cre-loxP system²⁹. Tissue-specific promoters drive expression of Cre recombinase, which then deletes any DNA flanked by loxP sequences³¹. Many prostate cancer GEMMs have been generated over the years (Table 2)^{21, 32–89}.

A drawback to using GEMMs is the length of time it takes to make them; it can take 6-12 months or longer to generate a model (depending on the number of genes being excised), and this is accompanied by the high cost of maintaining mouse colonies. New technology, the CRISPR-Cas9 system, has significantly reduced the time and cost, and it now can take a mere 4 weeks to generate a GEMM⁹⁰. But even with a shorter generation time, genetically engineered mice are typically aged out a long time relative to xenograft models. Another issue with GEMMs is that they rarely metastasize to bone, which is a much-desired aspect of a prostate cancer model, although some models have reported bone metastases⁴⁸. Even when metastases are present, mouse tumor cells are technically difficult to detect in normal mouse tissue, and the field is generally critical of reports of metastatic lesions as to whether or not the metastases are truly metastatic and are epithelial in origin⁹¹.

GEMMs have rarely been used for drug discovery. The TRAMP model (see Table 2) has been used to test antioxidants, anti-androgens, immune therapy, and other drugs^{92, 93}. In another example, rapamycin (an mTOR inhibitor) and PD0325901 (a mitogen-activated protein kinase – MAPK – inhibitor) were tested on the *Pten*^{+/-}; *Nkx3.1*^{ko} mouse model^{32, 94}. Two disadvantages to using a GEMM for drug discovery experiments such as these are that the mice often must be aged out for months before tumors consistently develop (this specific experiment waited 10 months), and that experimental groups are often smaller (n=4 in many of these experiments). The advantage of GEMMs is that they carry genetic modifications often associated with the human condition. Due to tissue-specific conditional models, these modifications can be made directly in prostate tissue in a cell-specific manner. This more closely resembles the human disease, in which mutations occur in the prostate cells themselves; this allows for a greater understanding of the molecular mechanistic causes of the disease. GEMMs are also useful for studying the stromal environment, as fibroblast-directed genetic modifications have been shown to drive prostate neoplasia³⁶.

2.3b Cell line xenografts

In cell line xenograft models, human cancer cells are injected into immune-deficient mice. Due to the number of available cell lines, genes that can be manipulated, sites of injection, backgrounds of immune-deficient mice, and imaging modalities, there is a myriad of experiments that can be done using xenografts⁹⁵. This model is used most often for drug studies, due to its reproducibility, quick experimental timeframe, and relative ease of experimental setup.

Several immune-deficient models exist: athymic nude⁹⁶, severe-combined immunodeficiency (SCID)⁹⁷, non-obese diabetic (NOD)-SCID⁹⁸, NOD-SCID-interleukin 2 receptor gamma null (NSG)⁹⁹, and recombination-activation gene (RAG)^{100, 101} (Table 3).

Of these, the NSG mouse is the most immune-deficient, and has the highest take rate of human immune cells and cancer cells^{29, 99}. The biggest issue with using immune-deficient mice for cancer research – even though they are required to grow human cancers – is that immune cells have been shown to play important roles in cancer progression and eradication^{102–104}. The immune system's roles in cancer therefore cannot be studied in immune-deficient mice. A final issue with this model is the relative lack of cell lines in prostate cancer¹⁰⁵. While other types of cancer have dozens of cell lines to choose from, the vast majority of prostate cancer cell line work is done in PC3¹⁰⁶, LNCaP and its derivatives^{107–109}, VCaP¹¹⁰, MDA PCa¹¹¹, and DU145¹¹² lines.

One potential way to study the immune system's effect on cancer and metastasis in an immune-deficient model is to “humanize” the mouse by injecting human hematopoietic stem cells or differentiated immune cells, followed by injection of human cancer cells. These mice have no endogenous immune system but have some aspects of a human immune system within them. They have been studied primarily in the immunology field and for tumor vaccines, as well as in other fields¹¹³. The hu-PB-NOD/SCID model in particular has been used to study immune response to prostate cancer and to develop prostate cancer vaccines^{114–116}. It would likely be beneficial to use these or similar mice not only for tumor immunology studies, but also for any xenograft study, as immune cells can affect tumor growth and metastasis. Some limitations of this model are incomplete hematopoietic differentiation, inadequate cellular interactions from perhaps under-developed immune cells, and limited production of human cytokines in mice¹¹³.

2.3c Patient-derived xenografts

A patient-derived xenograft (PDX) is generated through human tumor tissue being directly placed or injected into an immune-deficient mouse. This can be done using tissue obtained during surgery, or through needle biopsy at diagnosis¹¹⁷. Prostate tumors have been difficult to establish, as they require high quality tumor tissue and thorough characterization^{117–122}. While PDX models capture more of the genomic, epigenetic, and proteomic diversity within prostate cancer, it has been difficult to use PDXs for drug discovery¹²³. Even so, multiple institutions have now developed many novel PDXs, including the LuCaP models^{124–127}, the bone metastatic BM18 model¹²⁸, multiple models from MD Anderson^{129, 130}, and others^{119, 131, 132}. It is challenging to standardize therapeutic protocols since tumor tissue varies genetically and phenotypically between patients. It is believed, however, that PDXs represent the future of personalized or individualized medicine – each patient will some day be able to have their tumor grown as a PDX (or avatar) and analyzed for genomic alterations, identifying actionable targets for therapy¹²³. Lessons learned from the many existing and ever-increasing number of PDXs should facilitate the harnessing of this technology for novel biological discoveries at each stage of prostate cancer¹²².

2.3d Syngeneic models

In a syngeneic model, tumor tissue from one mouse is removed and placed into another mouse of similar genetic background and therefore is immunologically compatible. The major upside to this model is that the immune system remains intact, which solves the problem of using immune-compromised mice in xenografts. With these models, tumors can

be grown relatively quickly, which avoids the long timeline issue in GEMMs. However, syngeneic prostate cancer models are extremely rare due to the scarcity of mouse prostate tumors and cancer cell lines, and lack of effort being placed into generating immortal cell lines from mouse prostate cancer models. Some murine prostate cancer cell lines have been generated^{133–135}, and they provide new opportunities for novel drug discovery in mice with intact immune systems. A syngeneic PTEN-deficient model was used to develop and test novel tumor vaccines, using murine cell lines PTEN-P8 and PTEN-CaP8, derived from a PTEN knockout model. These cells were used to develop the vaccine, and then injected into wild-type C57BL/6 mice, where the vaccine was tested¹³⁶.

2.4 Using the mouse to test drugs

GEMMs are rarely used in drug studies, due to their lengthy time course, but they can be powerful models⁹². PDX and syngeneic models are also less often used for drug studies, although PDXs are becoming more popular. Syngeneic models are most often used in immunotherapy experiments due to the immune competency of the mice^{137–140}. Human cell line xenograft models are by far the most often used for drug studies. The most common method to test drugs currently is to subcutaneously inject prostate cancer cells, treat with drug(s), and then measure the change in subcutaneous tumor volume over time. Depending on the cell line involved, the subcutaneous model may also be used to study metastasis and a drug's effect on metastatic ability¹⁴¹.

Over the years, hundreds of drugs have been tested in mice to determine their efficacy in reducing tumor volume. Very few of these, however, have been translated into a drug to treat human cancer. In fact, only about 5% of anticancer compounds tested in animal models also show sufficient anticancer activity in phase III clinical trials and are ultimately approved by the FDA¹⁴². There are many reasons that could explain why tumors shrink in mice, but not in humans, using the same drug.

Subcutaneous cell line xenograft tumors grow in a foreign microenvironment with different host stroma than their native environment. Orthotopic injection models, in which prostate cancer cells are injected directly into the murine prostate, may better recapitulate the primary tumor's microenvironment^{143–147}, but are not as easy as the subcutaneous model to assess for growth and therapeutic effects.

The reproducibility that makes the cell line xenograft model so attractive for experimentation may also be confounding drug discovery and prognostic value. Lack of tumor heterogeneity may play a negative role in the ability to predict a drug's effectiveness in humans¹¹⁷. A drug that may cause a clonal cell line tumor to shrink may have little to no effect on human prostate tumors that are extremely heterogeneous.

Mice are approximately 300 times smaller than humans, resulting in different pharmacokinetics and pharmacodynamics of drugs. A mouse's average heart rate is approximately 600 beats per minute as compared to humans, whose average heart rate is 70–80 beats per minute. Mice in general can tolerate larger doses of drug because they can clear the chemicals from their system more quickly¹⁴⁸. In general, however, allometric scaling laws are believed to work to extrapolate mouse drug concentrations to humans¹⁴⁹.

The delivery mechanism of drugs may differ between mice and humans, depending on the drug's solubility, mechanism of action, and metabolism. Drugs in humans are generally either oral or intravenous. Administration of oral drugs is technically difficult in mice, making translation to humans challenging. Drugs are commonly given to mice via intraperitoneal injections, which mimics intravenous administration in patients. Drug metabolism can also vary due to the expression of different enzymes between species. The drug abiraterone, for example, varies in tissue-specific expression of 17 α hydroxylase (CYP17A1): it is expressed in the adrenal cortex as well as the gonads in humans, but only in the gonads in mice¹⁵⁰. This expression difference should be kept in mind, but research has still been done with abiraterone in xenograft models^{151, 152}.

There continues to be a notable lack of metastatic models in the prostate cancer field. Researchers often use pseudo-metastatic models, where cancer cells are injected directly into the heart or into the site of metastasis itself. These types of models bypass steps in the metastatic cascade, and therefore, drugs that are tested in these models are being measured against a simulated metastatic lesion, which is not representative of the metastases seen in human patients. Metastatic lesions are rarely found in GEMMs, possibly because metastases never actually develop, possibly because the primary tumor grows too quickly and the mice must be euthanized prior to development of metastases, or potentially because of immunosurveillance mechanisms. Metastases can be detected in xenograft models, however, and researchers should know which injection type to use, depending on the cell line⁹⁵.

Some of the differences seen in drug responses between mice and men may not be due to the differences between the species at all but may be due to the poor experimental design. Some clinical trials do not have enough statistical power to find the true benefit of a drug that showed promise in mice¹⁵³. Some clinical trials may give a drug later in a time course than was helpful in mice, thereby trying to have an effect too late. These two issues – not enough statistical power and bad timing – also plague the mouse studies as well. Other issues arise with experimental planning. There is innate bias in the interpretation of data that can be reduced with some quality control measures, such as blinding the study participants, randomizing the mice (or patients), and performing *a priori* statistical calculations to determine how many subjects are required. A study of 2,000 articles published between 1980 and 2000 in seven of the leading scientific journals (including *Science*, *Nature*, and *Cell*) discovered that very few studies actually use these quality control techniques¹⁵⁴. Guidelines on how to care for and utilize mice in cancer research settings have been published, however, which has led to better practices in animal research¹⁵⁵. Another study reviewed the animal model publications of stroke and concluded that a publication bias exists in which negative data is rarely published, and published articles generally report significant findings (a.k.a. positive data)¹⁵⁶. This bias may skew the way the field interprets the value of a novel drug or molecule. The number of variables to take into account when designing a preclinical trial in mice is underappreciated¹⁵⁷. Several recommendations have been published to improve the quality of preclinical research publications, including allowing for publication of negative data, increasing communication between physicians and scientists and patients, and increasing access to cancer research tools (such as mouse models) among research scientists¹⁵⁸. Table 4 discusses many practical questions to consider when planning a mouse project.

3. Conclusion

Metastatic prostate cancer is incurable. Experimental models are essential to discover novel therapeutic drug targets. The mouse is the best model organism for this purpose. On the organismal and prostate scales, mice have many similarities to humans. Of course, many differences also exist. These differences must be taken into consideration during the planning stages of any mouse experiment. Some experiments are not possible due to these differences, particularly in the realm of immunity. Shrewd planning and the development of innovative novel techniques have overcome many of these differences. Unfortunately, many new drugs work to shrink tumors in mice, but fail in human clinical trials. Many variables exist that could explain this phenomenon. Better planning, more quality control measures, and new technologies should help to increase the number of drugs that are translatable from mice to men.

4. Expert Opinion

The ultimate goal in prostate cancer research is to identify the underlying cellular and molecular causes of tumorigenesis, progression, castration resistance, and metastasis so that drugs can be developed to cure the specific stages of the disease. The mouse is not a perfect organism with which to complete this goal – however, a perfect model does not exist. Due to the numerous legitimate ethical issues with performing research on humans, model organisms such as the mouse will be necessary until better models can be developed.

The attrition rates between the discovery of a successful therapeutic agent in a mouse study and approval for human use remains appalling. The field suffers from use of clonal cell lines, lack of immunocompetent models that recapitulate human disease, lack of understanding of how mice metabolize agents, and lack of well-designed rigorous clinical trials in mice¹⁰⁵. The situation need not be as dire as it sounds. Though there is not a single set of instructions for every mouse experiment to work perfectly, there are certain rules, guidelines, and sensible practices that if followed would increase the probability of translatable data to be produced and bias to be reduced (Table 4 and ref. ¹⁵⁷).

Perhaps the biggest challenge in the prostate cancer field is the generation of an immune-competent mouse model that recapitulates the late stages of prostate cancer, particularly bone metastasis. Bone metastasis is what is killing prostate cancer patients, but we still lack experimental models with which to discover new molecules and drugs. Some GEMMs have reported bone metastasis, albeit infrequently, and these metastatic lesions should be validated and turned into cell lines that can be used for syngeneic models. Immune-deficient mice with a “humanized” immune system might also be useful in this purpose. These types of models hold much potential.

Several areas of research hold particular promise in the coming years. As the number of PDX models increases, there should be a wealth of information representing an ever-expanding population of prostate tumors. Though reproducibility will likely remain low due to the heterogeneous nature of prostate cancer, these experimental models are the most realistic of human cancer and should provide new directions and molecular strategies for

therapeutics. The CRISPR/Cas9 genomic modulation system holds incredible potential for swiftly advancing the number of GEMMs, thereby allowing for swifter validation of candidate genes as tumorigenic or metastatic. The decreased time and cost to make a conditional knockout or mice expressing a gene with a point mutation allows for much more flexibility than previous years and opens the doors to many more possibilities. Finally, as imaging technologies improve, *in vivo* detection of tumor size, disseminated tumor cells, and metastatic lesions will vastly improve our ability to test drugs in an efficient manner over longer time courses^{159–161}.

The future of prostate cancer research using mouse models is moving in the right direction. We are constantly gaining new knowledge and insight and are learning from past mistakes^{122, 158}. Hopefully, researchers will respect the differences that exist between mice and men and will move with care and caution, ensuring that our work is performed and recorded with quality control measures in place. The increasing number and quality of open access journals should also improve the amount of “negative” data available, so as to open up further lines of communication to determine the true value of a molecule or compound being used in anti-cancer studies. Free-flowing communication and information will be the key for the ultimate goal to be realized.

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Highlights

- Metastatic prostate cancer is incurable, and indolent prostate cancer is over-treated. More experimental models are needed, and the mouse is an ideal model organism.
- Mice and men have many similarities at the genomic and anatomic level, but also many differences. These differences should be considered when planning, interpreting, and reporting results.
- Four types of prostate cancer models exist: genetically engineered mouse models (GEMMs), cell line xenografts, patient-derived xenograft (PDXs), and syngeneic models.
- Very few drug studies conducted in mouse models have produced effective drugs for human use.
- Mouse-human biological differences, tumor stroma differences, lack of tumor heterogeneity and immune cells in mouse models, poor clinical trial design, and publication bias could explain this attrition rate.

Figure. Diagram of mouse and human prostate anatomy and histology

Only one of each lobe is represented in the mouse prostate anatomy diagram. Protein markers that are commonly associated with each type of prostate cell are listed beneath the cell types. Adapted from references^{162, 163}.

Table 1

Types of cancer mouse models

Model Type	Brief description	Advantages	Disadvantages
Genetically engineered mouse model (GEMM)	Target genes are knocked in or out in mouse cells (either whole body or tissue-specific), causing a phenotype	<ul style="list-style-type: none"> • Intact immune system • Studies genes that are deregulated in human disease • Cancer generally originates from organ of interest, and generally looks histologically similar to human cancer 	<ul style="list-style-type: none"> • Difficult to model metastasis • Variable tumor growth • Can inadvertently introduce off-target phenotypes • More costly and time-consuming than xenograft models
Cell line xenograft	Human (or other) cell lines are injected into immune-deficient mice	<ul style="list-style-type: none"> • Well-characterized cell lines available • Ability to swiftly study various properties of cells and genes, such as tumor growth or metastatic capacity • Multiple potential injection sites to answer many types of questions • Relatively reproducible 	<ul style="list-style-type: none"> • Unable to study or account for immune system due to immune-deficiency of host • Tumor-stroma interaction is likely not accurately recapitulated, as foreign cells are introduced
Patient- derived xenograft (PDX)	Human (or other) tumor tissue removed from patient is injected into immune-deficient mice	<ul style="list-style-type: none"> • Tumor tissue taken directly from tumor source, which should more accurately recapitulate a tumor microenvironment and genetic abnormalities than cell lines grown in culture 	<ul style="list-style-type: none"> • Unable to study or account for immune system due to immune-deficiency of host • Tumor- stroma interaction is likely not accurately recapitulated, as foreign cells are introduced • Reproducibility is low
Syngeneic model	Mouse tumor tissue is injected into immune-competent mice of the same genetic background	<ul style="list-style-type: none"> • Intact immune system • Relatively reproducible • More accurate tumor-stroma interaction than in human xenografts • Can use GEMM or chemically-induced cancer models 	<ul style="list-style-type: none"> • No human cells are involved • Tumor-causing genes may not share features of human genes

Table 2

Genetically engineered mouse models of prostate cancer

Hyperplasia or prostatic intraepithelial neoplasia (PIN)	Carcinoma, no metastasis reported	Carcinoma, metastasis reported
<p>p27 KO [50, 58, 70] Mt-PRL [84] Pten^{+/-} [45] Nkx3.1 KO [35] PB-mAR [77] PSA-Cre;Nkx3.1-flox [33] PB-FGF8b [76] PB-Akt [67] PB-FGFR1 [52] MMTV-Cre;LSL-Catnb^{lox(ex3)} [37] MMTV-Cre;Pten-flox [34] FSP1-Cre;TGFβ-flox [36] PB-Cre;Rb-flox [65] PB-Ras [75] PB-Cre;LSL-Ph15LO-1 [56] PB-Cre;IGF1-flox [78] PB-Erg [60] Pten × p53 KO [43] PB-Cre;LSL-Catnb^{lox(ex3)} [85] PB-Cre;Brca2-flox;p53-flox [51] PB-PI3K p110B [61] PB-Cre;Pten-flox;CAG-Sox9 [79] PB-Cre;Scrib-flox [72] Nkx3.1-CreERT;Apc-flox [80]</p>	<p>BK5-IGF1 [46] Pten × p27 KO [44] PB-myc [21] PB-Cre;Pten-flox;p53-flox [42] PB-neu [62] PB-Cre;Apc-flox [38] PB-Vav3 [63] PB-Akt × p27 KO [66] Pten^{+/-} × Par4 KO [49] PB-Cre;LSL-Catnb^{lox(ex3)};LSL-Kras^{V12} [73] Nkx3.1-CreERT;Pten-flox [83] PB-Erg × Pten^{+/-} [40] PB-hepsin;PB-myc [71] Pten^{+/-} × Phlpp1 KO [41] PB-Cre;Scrib-flox;LSL-Kras^{G12D} [72] PB-Cre;Pten-flox;p53-flox;LSL-cMyc [57] PB-Cre;p53-flox;Smad4-flox [48] PB-FGFR1 × H2Kb-iLrp5 [39]</p>	<p>C3(1)-Tag [68] * PB-Tag (TRAMP) [55] * FG-Tag [74] * LPB-Tag (LADY) [89] * CR2-Tag [54] PSP94-Tag [53] LPB-Tag;PB-hepsin [59] Pten × Nkx3.1 KO [32] PSA-Cre;Pten-flox [64] PB-Cre;Pten-flox [82] PB-Cre;p53-flox;Rb-flox [87] PB-FGF8b × Pten^{+/-} [86] PB-Cre;Pten-flox;Smad4-flox [47] * PB-Cre;Pten-flox;p53-flox;Smad4-flox [48] * PB-Cre;Pten-flox;p53-flox;LSL-Tert [48] PB-Cre;Pten-flox;LSL-Kras^{G12D} [69] Nkx3.1-CreERT;Pten-flox;LSL-Braf^{V600E} [81]</p>

* Bone metastasis reported

Reference numbers in brackets [].

Table 3

Types of immune-deficient mice

Type	Gene(s) affected	Immune cells that are deficient or defective
Athymic nude	Forkhead box N1 (Foxn1)	T cells
SCID	Protein kinase D1 (Prkd1)	T and B cells
NOD-SCID	Cytotoxic T-lymphocyte-associated protein 4 (Ctla4) & Prkd1	T, B, NK, and complement cells
NSG	Interleukin 2 receptor gamma (Il2rg) & Prkd1 & Ctla4	T, B, and NK cells, macrophages, and dendritic cells
RAG	Recombination activating genes 1 or 2 (Rag1 or Rag2)	T and B cells

Table 4

Practical questions to consider when planning a mouse experiment

<ul style="list-style-type: none"> • Does the question you are asking require a mouse model? What is the cost/benefit analysis of doing a mouse experiment versus other types of experiments? • What is your institution's policy on mouse work? Do you have IACUC approval? How long will that take? • Will the disease you wish to study be accurately recapitulated in mice? Is there already a model of the disease that exists? • Is the molecule you wish to study analogous to humans? If it is a gene, is its expression analogous to humans? If it is a ligand or receptor, do they behave similarly in mice and humans? • What type of mouse model would you need to answer your question? • Is the immune system an essential aspect of your study? • What genetic background of mouse is most appropriate? • How many mice are needed for the results to be statistically and biologically significant? • How will you reduce unintentional bias? (Examples of solutions: blinding, randomization, <i>a priori</i> sample size calculation). 	<ul style="list-style-type: none"> • What are the measurable outcomes? How will you report the data? How will you image the mice? Will you collect blood, tissue, etc? Are you measuring tumor growth specifically, or will you also assess invasiveness, metastasis, etc.? • What is the timeline of the experiment? Is the mouse of an appropriate age, and will the mouse be euthanized at a specified time, or allowed to live until pre-determined criteria are reached? • If doing a drug study, what is the half-life of the drug? How will you administer the drug? How will you measure toxicity? How will you measure the effectiveness of the drug? • If using a conditional knock-out model, is the Cre you are using leaky in other tissues? Will there be off-target effects? • If using a xenograft model, what type of injection will appropriately answer your question (IV, SC, etc.)? How will you manipulate gene expression so that it remains stable in the mouse (RNAi, CRISPR/Cas9, etc.)?
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