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Best Practices for Preclinical In Vivo Testing of Cancer Nanomedicines

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Significant advances have been made in the development of nanoparticles for cancer treatment in recent years. Despite promising results in preclinical animal models, cancer nanomedicines often fail in clinical trials. This failure rate could be reduced by defining stringent criteria for testing and quality control during the design and development stages, and by performing carefully planned preclinical studies in relevant animal models. This article discusses best practices for the evaluation of nanomedicines in murine tumor models. First, a recommended set of experiments to perform is introduced, including discussion of the types of data to collect during these studies. This is followed by an outline of various tumor models and their clinical relevance. Next, different routes of nanoparticle administration are overviewed, followed by a summary of important controls to include in in vivo studies of nanomedicine. Finally, animal welfare considerations are discussed, and an overview of the steps involved in achieving US Food and Drug Administration approval after animal studies are completed is provided. Researchers should use this report as a guideline for effective preclinical evaluation of cancer nanomedicine. As the community adopts best practices for in vivo testing, the rate of clinical translation of cancer nanomedicines is likely to improve.

1. Introduction

Cancer nanomedicine is a rapidly growing field, with publications on Web of Science soaring from just 74 in 2008 to 952 in 2018.[1] As the discipline expands, it is important for researchers to consider the rigorous testing that must be performed in order for nanoparticles to progress from in vitro and in vivo studies in the lab to human use in the clinic. Typically, nanomedicine development begins with in vitro characterization of the materials prior to in vivo testing. However, even if nanoparticles succeed in preclinical testing, there is a dauntingly low success rate of cancer nanomedicines that make it through clinical trials. In a recent survey of the translation of cancer nanomedicines by Schwendeman and co-workers, it was noted that the success rate of 94% in phase I clinical trials drops dramatically to 48% in phase II and even further to 14% in phase III.[2] Given these dismal translation rates despite success in preclinical testing, better ways to predict the safety and efficacy of nanomedicines in human subjects are needed.

In vitro and in vivo data correlation (IVIVC) is a form of predictive mathematical modeling used during pharmaceutical development that allows researchers to correlate an in vitro property with a relevant in vivo response. The goal of IVIVC is to reduce development time and facilitate optimization of drug formulations.[3] Using a tool such as IVIVC to evaluate nanomedicines may improve the clinical success rate. Dobrovolskaia et al. have reviewed in vitro assays that have "good" IVIVC, which include hemolysis, complement activation, cytokine release, and opsonization and phagocytosis assays.[4,5] Implementing these assays to test nanomedicines may improve clinical translation. Alternatively, using more complex in vitro models of cancer to evaluate nanomedicines may improve the correlation between in vitro studies, in vivo findings, and human results. There has been rapid progress in recent years toward creating more effective in vitro models of cancer,[6,7] including the development of systems that can mimic tumor dormancy and reactivation[8] and the development of systems that can mimic site-specific metastasis.[9,10] Indeed, organ-on-a-chip technologies may prove useful for evaluating the therapeutic efficacy of nanomedicines in the future. However, even with these advanced in vitro models, which are still years from implementation as drug/nanomedicine-screening tools, it will be difficult to determine how well a nanoparticle will translate into higher order animals, especially when the immune system is

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introduced. Between 1969 and 2005, 10–20% of all drugs that were withdrawn from use in the clinic were removed because of immunotoxicity.[4] Accordingly, systems and animal models that can reveal the immune response to nanomedicines are a critical component of preclinical testing that must be included in the development process.

In part due to the complexity of the immune system, even with extensive in vitro testing and characterization, different animal models and ultimately human subjects will react unpredictably to administered nanoparticles. Thus, there has been a recent movement toward entering in vivo studies earlier on in the development process. If the progression to in vivo testing is done without thorough in vitro vetting, researchers must be extra cautious of animal welfare. It is strongly recommended that, before progressing to animal studies, researchers should, at minimum, perform extensive characterization of the nanoparticles and ensure the formulation is reproducible with limited batch-to-batch variability. For a more in depth review of proper nanoparticle characterization, readers should turn to Faria et al.[11,12]

In the following pages, this article will summarize best practices for preclinical evaluation of cancer nanomedicines using murine tumor models. The topics discussed range from experimental setup and types of tumor models to animal welfare and clinical relevance. The goal of this report is to inform researchers about various aspects of evaluating cancer nanomedicines in vivo, with the hope of improving the reliability of results and enhancing the rate of success as technologies move from the lab to the clinic.

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2. Types of In Vivo Studies to Perform to Evaluate Cancer Nanomedicines

When evaluating nanoparticles in vivo, it is important to have a thorough experimental design and data analysis plan in place prior to beginning testing. In general, preclinical work should include maximum tolerated dose (MTD) and safety studies, pharmacokinetic or absorption, distribution, metabolism, and elimination (ADME) studies, and efficacy and mechanism of action studies (**Figure 1**). To reduce animal morbidity, an MTD study should be performed prior to ADME studies and efficacy studies. Each of these experiments are described in further detail in the following sections.

2.1. Maximum Tolerated Dose and Safety Studies

MTD studies are used to determine the highest dose of drug that can be administered without inducing unacceptable side effects or overt toxicity within a specified period of time.[13] MTD studies

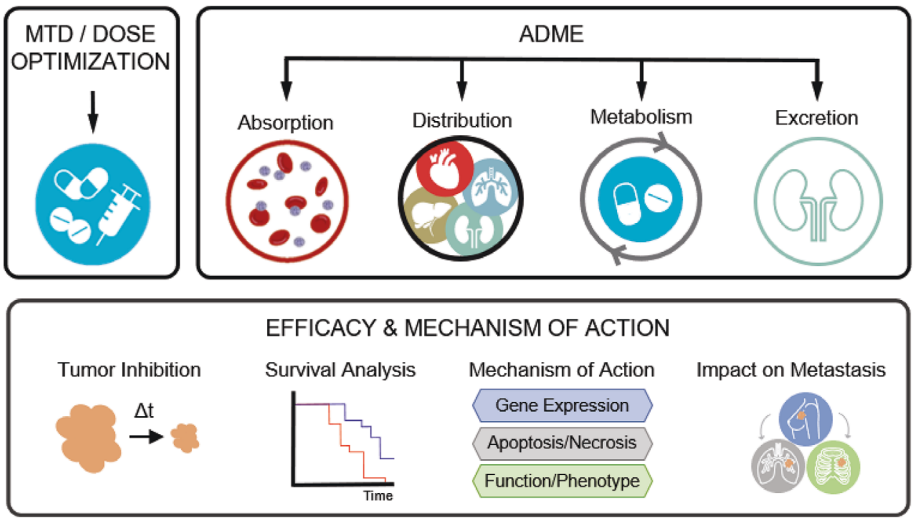

Figure 1. Overview of animal studies used to preclinically evaluate cancer nanomedicines. Maximum tolerated dose (MTD) and safety studies allow for dosing optimization while ADME studies define the absorption, distribution, metabolism, and elimination of therapeutics. Efficacy studies primarily aim to elucidate the impact of treatments on tumor growth and animal survival and are supplemented by studies to confirm the predicted mechanism of action of the therapy. In cases where treatments are designed to attack metastasis, the size and number of metastatic lesions may be measured following treatment to confirm efficacy.

may be performed in nontumor bearing animals and frequently consist of short duration dose escalation and dose ranging studies that are performed while monitoring acute toxicity. Side effects noted during these studies are classified as mild, moderate, and substantial. Mild side effects typically manifest as reduced weight gain. Weight loss up to 20% is more serious and considered a moderate side effect. In the extreme, unresponsiveness is classified as a substantial side effect. Moderate and substantial side effects are commonly used end points indicating a dose-limiting toxicity (DLT). When DLTs are experienced by a specified percentage of the treatment group, the previous dose is denoted as the MTD. It is important to note that the MTD can vary by animal strain,[14] so this type of study should be performed with every model used. Researchers should also consider that the addition of supportive treatments like dexamethasone, which is used in clinical trials to reduce inflammation and infusion-related adverse events, may alter the MTD.[14]

Determining the MTD is an important first step because it provides the maximum opportunity to detect chronic adverse effects and other such hazards of a given nanomedicine. By investigating the MTD first, researchers can minimize animal morbidity in future studies.[13] The determined MTD should subsequently be used for the pharmacokinetic and pharmacodynamic studies described below as well as for long-term safety assessments.

Besides determining MTD, researchers should also assess a number of other parameters to characterize the safety of a nanomedicine. These safety analyses may be performed in healthy animals, or they may be performed in tumor-bearing animals once the MTD and effective therapeutic dose have been identified. Safety studies should include analysis of serum cytokines (e.g., interleukin-6 (IL-6) and IL-12), blood chemistry (i.e., counts of red blood cells, white blood cells, etc.), and liver enzymes (e.g., alanine aminotransferase, aspartate aminotransferase, etc.) in both mice treated with the therapeutic nanomedicine and control mice (those treated with saline or nontherapeutic nanoparticles). Additionally, each animal's general health should be monitored from the start of every in vivo study. In particular, the weight of the animal should be measured beginning at the day of tumor inoculation and continuing daily (or at least several times per week) until the time of euthanasia. Weight loss can indicate either that the tumor burden has become too great or that the nanoparticles are eliciting undesirable side effects, as noted previously for DLTs. Including comparisons to appropriate control groups, which are discussed in Section 4.2, will help reveal if reformulation of the nanomedicine or the dosing scheme is required.

2.2. ADME Studies

After determining the MTD, researchers should proceed to evaluate the pharmacokinetics of nanomedicines. ADME studies provide important pharmacokinetic information like drug clearance, bioavailability, exposure time, half-life, and distribution volume.[6,15] Absorption, or bioavailability, studies evaluate how much of the nanomedicine enters the bloodstream and how quickly this occurs.[15] This is often investigated by examining plasma concentration of the nanomedicine's cargo. Distribution studies are used to determine the rate of accumulation of nanoparticles in various organs and their tissue specificity.[15] In cases where the nanoparticles do not have inherent optical properties to support their visualization with an in vivo imaging system (IVIS), fluorophores can serve as substitutes for the therapeutic cargo or be used to tag the therapeutic cargo, allowing the investigator to determine the ultimate fate of the nanomedicine. Caution must be taken when using this approach, as it is not guaranteed that the fluorophore or fluorophore-labeled cargo will distribute in the same manner as the unmodified agent. As an alternative to fluorescence-based analysis or HPLC (which is commonly used to examine drug distribution), researchers could use inductively coupled plasma-mass spectrometry (ICP-MS) or neutron activation analysis (NAA) to analyze nanoparticle content in various tissues. This is most commonly employed for metal-based nanoparticles like gold.[16,17] Finally, metabolism and elimination studies frequently require analyzing blood chemistry throughout treatment cycles. Metabolism experiments evaluate how fast and through what mechanism metabolites are formed and whether these metabolites are active or toxic in any way.[15] These are currently rarely performed in preclinical testing of nanomedicines, but their implementation may provide critical insight that will allow researchers to address any toxicity issues early in development. Elimination studies primarily investigate the nanoparticles' interaction with the immune system and determine the route and speed at which they are cleared from the body.[5,15] This information is important, as unexpected immune responses are responsible for many failures of nanomedicines in the clinic.

2.3. Pharmacodynamic and Efficacy Studies

In addition to performing MTD and ADME studies, researchers should also evaluate the pharmacodynamics and efficacy of the treatment. Pharmacodynamics is the study of the relationship between the concentration of a therapy and its effect on the body and is derived from the Greek words "pharmakon" and "dynamikos," which mean "drug" and "power," respectively. The goal of these studies is to identify the dosing regimen that induces the maximum therapeutic effect. The type of data that should be collected during these studies is impacted somewhat by the type of treatment that is facilitated by the nanomedicine (drug delivery, gene regulation, phototherapy, immunotherapy, etc.). Below, a summary of the type of data that is important to collect during efficacy studies is provided. Readers should note that this list is not all-inclusive, and studies must be designed to maximize the information gained for the particular therapy and disease being evaluated.

Tumor size is the primary metric that must be collected for each animal from the time of tumor formation until study completion. Depending on the tumor location, its size may be measured with Vernier calipers or by imaging techniques. If the tumor cells are fluorescent or bioluminescent, then an IVIS may be used to monitor tumor growth; otherwise, other imaging tools (e.g., MRI, CT, and photoacoustics) may be employed to track tumor growth. Upon study completion, datasets of tumor size versus time can be plotted for individual mice or presented and evaluated as averages for each treatment group to compare growth rates. Another metric that is important to track in efficacy

studies is time-to-event data (such as survival time or time until tumor diameter reaches 10 mm). After plotting these data in Kaplan–Meier curves, a log-rank test may be performed to analyze differences in survival between two or more independent groups.[18]

Besides the metrics of tumor size and animal survival, other information may be important to collect depending on the goal of the treatment. For example, if a treatment is designed to suppress metastasis, then the number and size of metastatic nodules that form will be important to monitor. Likewise, if a treatment aims to promote antitumor immunity, then tumor rechallenge studies should be performed. Ultimately, researchers must take initiative to be informed of the appropriate endpoints to analyze for their particular therapeutic approach. To supplement information regarding efficacy, researchers should also collect data that may provide insight to a nanomedicine's mechanism of action. This may include analysis of intratumoral gene expression, analysis of immune cells and cytokines, and more. The more information that can be gained from preclinical animal studies, the more likely it is that therapeutic success in humans will ultimately be achieved.

As a note of caution, it is important to highlight that while it is exciting for researchers to observe preclinical success when testing their nanomedicines in murine models, it is imperative to not overstate such results. As the field has seen on multiple occasions, statistically significant results from preclinical animal studies may not always translate to human clinical success. In fact, clinical success is the exception rather than the rule. Noting the limitations of the animal study (e.g., simplicity of the tumor model versus human scenario, differences in immune system between mouse and human, etc.) when presenting results is important to prevent overhype. Scientists must present their findings to the public in a responsible and accurate manner, as this will not only allow other researchers to advance more quickly upon the findings, but also prevent patients from gaining premature hope when a treatment is still years or decades away from the clinic.

Section 2 has emphasized the types of studies that should be performed when evaluating cancer nanomedicines in murine models. In Sections 3 and 4, some factors one must consider when planning animal studies are discussed, including the types of tumor models available for research (Section 3), the route of nanoparticle administration (Section 4.1), and the inclusion of proper control groups (Section 4.2).

3. Factors to Consider When Designing Animal Studies: Available Murine Tumor Models

Mice have long been the model of choice in preclinical drug development due to their similarities with humans at the genetic level and their ease of handling, which simplifies study execution. However, cancer is a complex, multifactorial disease and one mouse model cannot recapitulate the entirety of human cancer progression from primary tumor development to metastasis. Moreover, a single mouse model cannot capture the genetic, epigenetic, immunological, histopathological, and structural heterogeneity of cancer.[19–21] Therefore, researchers should evaluate nanomedicines in multiple murine models before moving to higher order species (as funding allows). The following sections and **Figure 2** summarize various mouse models that are available for preclinical testing of nanomedicines. Models are divided based on tumor location and the source of the tumor cells.

3.1. Tumor Models Described by Location

3.1.1. Subcutaneous Tumor Models

Subcutaneous tumor models are the most frequently utilized model to study the therapeutic efficacy of new anticancer agents. Xenograft models use human cancer cells injected subcutaneously in immunodeficient mice (athymic nude) or severely combined immune deficient (SCID) mice, while syngeneic models use murine cancer cells implanted subcutaneously in immune competent mice (Figure 2A). Various types of immunodeficient mouse models with their advantages and shortcomings are reviewed elsewhere.[22,23] Some advantages of subcutaneous tumor models include their relatively high take rates, robustness, and reproducibility versus other tumor models.[24,25] Also, since many of the cell lines used to form subcutaneous tumors have been examined in preclinical studies for decades, there is an abundance of baseline data associated with these models. The location of these tumors is a key advantage as well. Tumor growth can be monitored using simple caliper measurements, which speeds analysis and makes this model accessible to researchers who lack access to advanced imaging systems. Further, these tumors can be easily excised at desired endpoints to analyze the accumulation of a nanomedicine within the tumor and study its impact on tumor biology. Due to these numerous benefits, subcutaneous tumor models are widely used to determine nanoparticle dosing schedule, biodistribution, and therapeutic efficacy. It should be noted, though, that subcutaneous tumor models have limitations as well. They generally do not accurately represent the interaction of tumor cells with local stroma and microenvironmental factors due to the simple method of cell injection. Further, immunodeficient mice (which are typically used to form subcutaneous tumors) are costly and preclude complete analysis of the body's response to treatment. More advanced tumor models may better predict the safety and efficacy of nanomedicines in humans.

3.1.2. Orthotopic Tumor Models

In orthotopic tumor models, cancer cells are injected at the location where they would be present in human subjects. For instance, breast cancers would be injected in the mammary fat pad (Figure 2B) and glioblastomas would be implanted in the brain. Compared to subcutaneous models, orthotopic tumor models more closely simulate the natural tumor microenvironment and presence of supporting cells. However, their location at sites that are not amenable to caliper measurements requires that researchers use various imaging techniques to monitor their growth, and this may be challenging for researchers at institutions that lack imaging resources.[26] Orthotopic tumor growth

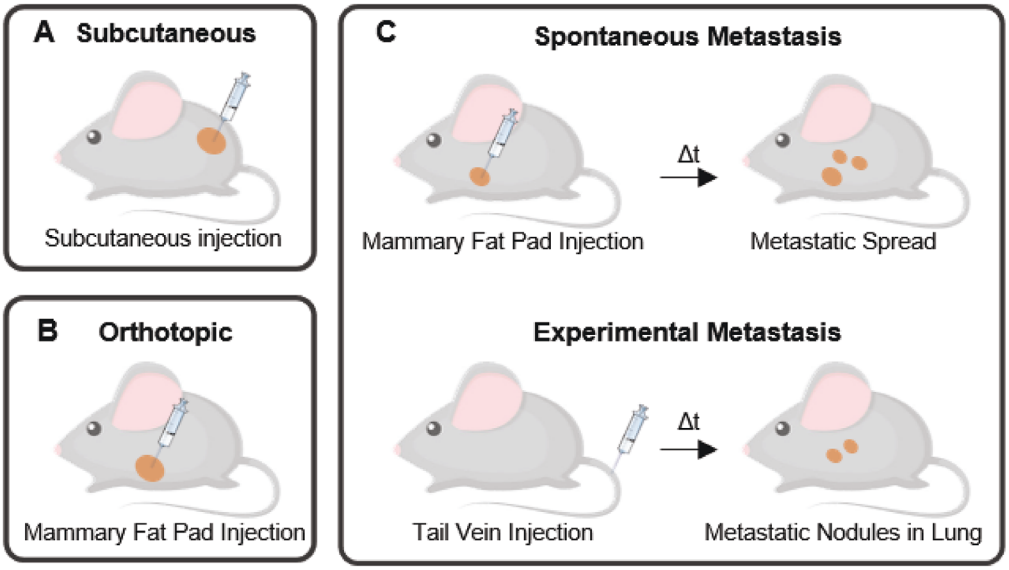

Figure 2. Available tumor models to evaluate nanomedicines. A) Subcutaneous tumors are formed by subcutaneous injection of cancer cells, typically at the flank region. These models offer a simple and cost-effective system for initial evaluation of nanomedicines. B) Orthotopic tumor models are produced by implanting cancer cells at the relevant tissue site (e.g., breast cancer cells would be implanted in the mammary fat pad). These models are more difficult to produce, but better replicate the physiology of human cancer than subcutaneous tumor models. C) Metastatic tumor models consist of spontaneous models and experimental models. In spontaneous models, cancer cells are implanted subcutaneously or orthotopically, and then disseminate to distant organs. In experimental models, cancer cells are delivered intravenously, leading to formation of metastatic nodules at sites such as the lung. Spontaneous models can recapitulate the entire cascade of events from primary tumor formation to metastasis but are slower to grow and more challenging to use than simple experimental metastasis models. Researchers must consider the benefits and drawbacks of these models and choose those that will best inform the development of their nanomedicine. Portions of this figure were produced with permission using Servier Medical ART templates, which are licensed under a Creative Commons Attribution 3.0 Unported License from Servier Medical Art; https://smart.servier.com.

is most commonly monitored by using whole body fluorescence or bioluminescence imaging to track the signal from cancer cells that have been engineered to express reporter proteins such as green or red fluorescent protein (GFP/RFP) and luciferase.[27–29] It is important that the expression of these reporters is stable in the cells over time, otherwise researchers may falsely conclude that a tumor is regressing. One of the drawbacks of orthotopic models is that the surgeries associated with their generation are more complicated than simple subcutaneous injections, which leads to lower tumor take rates and potentially fewer animals per study. This can be overcome by performing inoculation surgeries in larger numbers of mice, accounting for the lower take rate.

3.1.3. Metastatic Tumor Models

Although subcutaneous and orthotopic tumor models are valuable for determining the impact of nanomedicines on primary tumors, most cancer deaths are attributed to metastasis. Metastasis is a multistep process in which primary tumor cells invade surrounding tissue, enter vasculature, transport through circulation, and then extravasate into distal organs to form secondary tumors. Metastatic mouse models can be divided into two types: spontaneous and experimental (Figure 2C). In spontaneous metastasis models, primary cancer cells transplanted subcutaneously or (preferentially) orthotopically grow and metastasize into distal organs.[30] These models recapitulate the entire cascade of events from primary tumor formation to metastasis and are therefore best suited to study the effect of therapeutic agents on preventing metastasis formation. Many investigators have utilized the B16F10 murine melanoma metastasis model or the 4T1 murine breast cancer metastasis model to study anticancer agents.[31,32] However, it takes a few weeks to months for metastatic lesions to appear and only certain types of primary tumors can form metastases. Additionally, in some cases, the primary tumor must be removed surgically in order to study metastasis because if the primary tumor was not removed its burden on the animal would become too great.[33]

Experimental metastasis models are a simple alternative or supplement to spontaneous metastasis models. In experimental metastasis models, tumor cells are injected as a single cell suspension via the tail vein or via intracardiac injection (Figure 2C). Tail vein injections result in the formation of lung metastases and cells injected via the carotid cardiac route lead to bone or brain metastasis.[30] These models form metastatic lesions within a few weeks, which enables rapid evaluation of a treatment's ability to prevent metastasis formation or eliminate existing metastases. One limitation of these models, though, is that they do not recapitulate the initial steps in the metastatic cascade. The importance of this drawback depends on the mechanism of action of the nanomedicine being developed and should be considered by researchers when selecting which model to employ.

3.2. Tumor Models Described by Source of Cells

3.2.1. Cell-Line-Derived Tumor Models

One of the simplest and most convenient ways to form tumors or metastases in mice is to inject cells from human or murine cancer cell lines. These cell lines have typically been grown in laboratories in 2D culture and divided over many passages. These models are the least technically challenging and most affordable and can thus be established in a wide variety of laboratory settings. However, they utilize relatively homogeneous populations of cells, and thus fail to capture the heterogeneity of true human tumors. Another limitation is that cell lines grown in 2D monolayers for extended periods of time may acquire genetic and epigenetic features that differ from the cells originally collected from the patient. This altered phenotype, coupled with the lack of heterogeneity, makes tumor models that rely on cell lines less useful in terms of their pathophysiological relevance and ability to predict clinical outcome.[33] More advanced models, such as those described below, may be more reliable for evaluating nanomedicines and predicting patient response in the clinic.

3.2.2. Patient-Derived Xenograft (PDX) Models

In PDX models, pieces of tumors that were surgically removed from patients are transplanted subcutaneously in mice to better mimic tumor heterogeneity and architecture. PDX models preserve many desired features of primary human tumors, such as growth kinetics, histological and molecular heterogeneity, and stromal composition. Due to substantial improvements in accessing clinical samples and performing transplantation surgery, it has become simpler to establish PDX models. The transplant take rates for some cancers is as high as >75%, although it can be lower and the process is slow.[33] If researchers are not located near a hospital with access to patient tumor specimens, PDX models are now available for purchase from vendors such as The Jackson Laboratories and Charles Rivers. As PDX models capture the heterogeneity and complexity of human tumors, they are useful tools for screening new therapies. As these models become increasingly accessible and less expensive, they will become a valuable tool for the preclinical evaluation of nanomedicines.

While PDX models are usually implanted subcutaneously, they can also be located orthotopically. This adds another level of technical complexity, and requires that response to treatment be monitored by expensive and laborious imaging techniques as noted before. Therefore, most work with PDX models utilizes subcutaneous implantation. As a final note in this section, because PDX models require the use of immunocompromised mice, they are not well suited to evaluate the therapeutic efficacy and tolerability of immunotherapies that require activation of the immune system.

3.2.3. Genetically Engineered Mouse Models (GEMMs)

GEMMs address many of the challenges associated with cell line-based and PDX tumor models. They provide complete representation of cancer development in which a tumor grows spontaneously in the proper microenvironment of an immunocompetent mouse, maintaining the critical histopathological, molecular, and genetic features of the cancer. Accordingly, novel molecular entities can be better evaluated in GEMMs where dynamic processes such as the interaction of immune cells with the local tumor microenvironment are at play. Indeed, GEMMs are the only preclinical models currently available that can effectively evaluate immunotherapies.[34] Unfortunately, GEMMs are the most difficult model; they are slow to develop, and often require a deep understanding of cancer biology and genetics when designing studies and interpreting data. Another limitation of GEMMs is that tumors arise due to the mutation of a single potent oncogene, which is in contrast with the multiple genetic and epigenetic changes that occur during human tumor growth, progression and metastasis. While GEMMs can technically provide a model to study metastasis inhibition capabilities of a nanomedicine in the presence of a host immune system, it can be cumbersome to perform these studies due to the extremely slow rate of metastasis formation in these models. To overcome these limitations, tissue specific GEMMs with better temporal and spatial control of gene regulation, as well as germline and nongermline GEMMs have been developed, as reviewed in literature.[33,35–37]

Overall, there are numerous murine models for researchers to choose from when evaluating nanomedicines. Although not discussed here, humanized mice are another emerging resource that may be a valuable tool for cancer nanomedicine.[38] Researchers must carefully consider the advantages and disadvantages of each model presented in this article, and select the model(s) that will be most informative and predictive of clinical outcomes. In Section 4, other design considerations for animal studies are discussed, including the route of nanoparticle administration and proper experimental control groups, in order to aid researchers in collecting the most useful information from their studies.

4. Factors to Consider When Designing Animal Studies: Route of Nanoparticle Administration and Experimental Control Groups

4.1. Route of Nanoparticle Administration

Multiple routes of administration may be used to deliver nanoparticles to animal and human subjects. Since the route of administration will alter nanoparticle accumulation in the targeted area,[39] researchers must carefully select the mode that is most appropriate based on the planned clinical use of the material. The various advantages and disadvantages of oral, pulmonary, transdermal, and intravenous (i.v.) delivery methods are reviewed elsewhere.[40] This section focuses on the following administration methods as they apply to nanomedicine: i.v., intraperitoneal (i.p.), subcutaneous or intramuscular, and intratumoral (**Figure 3**).

4.1.1. Intravenous Injections

Intravenous injections are the most common route of nanoparticle administration and should be utilized in preclinical animal

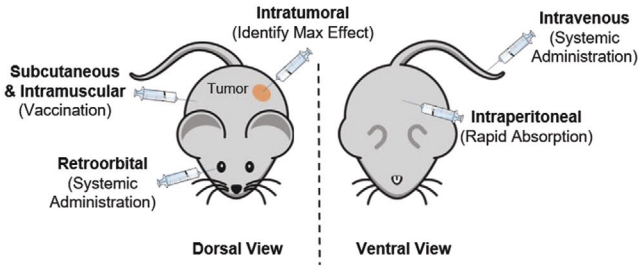

Figure 3. Routes of nanoparticle administration. When designing animal studies to evaluate cancer nanomedicines, researchers should utilize the most clinically relevant route of administration. Different administration routes and their utility are summarized in this figure. Portions of this figure were produced with permission using Servier Medical ART templates, which are licensed under a Creative Commons Attribution 3.0 Unported License from Servier Medical Art; https://smart.servier.com.

studies if this is how the nanomedicine will be applied in the clinic. There are two major types of i.v. injection: tail vein and retro-orbital (Figure 3). Tail vein injections can be technically challenging and typically require a restraining device and advanced warming of the animal to dilate the blood vessels.[41] In cases where tail vein injections are difficult (e.g., neonatal mice), retro-orbital injections are an acceptable alternative.[42] The animal must be anesthetized for this procedure, so proper animal care is critical (described in further detail in Section 5).

4.1.2. Intraperitoneal Injections

Intraperitoneal injections (Figure 3) are the least common route of nanoparticle administration, as they have limited relevance to the clinic. A portion of the dose from i.p. injections will enter the gut, abdominal fat, and other subcutaneous tissues.[43] The dose remaining in the peritoneal cavity will diffuse into surrounding tissues and be rapidly absorbed, but it will be subjected to first-pass elimination by the liver.[44] While i.p. injections can be used to administer anesthesia, they are generally considered unreliable for evaluating cancer nanomedicines. Accordingly, researchers should use this route only if there is substantial scientific justification.

4.1.3. Subcutaneous and Intramuscular Injections

Subcutaneous and intramuscular injections (Figure 3) are most commonly used for cancer nanomedicines that aim to stimulate the immune system. These injection methods allow nanoparticles to be rapidly absorbed into the bloodstream and can promote prolonged half-life,[45] making them promising alternatives to i.v. injections if the nanomedicine is irritating to the veins.[46] Additionally, immune cells in subcutaneous regions can collect therapeutics from the injection site and traffic them to lymph nodes, making subcutaneous injections ideal for nanovaccines.[47] In humans, intramuscular injections are preferable to subcutaneous injections because they enable more rapid absorption. Muscles not only provide a larger blood supply, but also hold a larger injection volume than subcutaneous tissues.[46] However, as discussed further in Section 5, intramuscular injections are not recommended in mice, which hinders translation of nanomedicines that rely on this administration method.

4.1.4. Intratumoral Administration of Nanomedicines

Intratumoral injections (Figure 3) involve the direct administration of nanoparticles into a tumor bed. While intratumoral injections may be used to identify the "maximum effect" that a specific dose of a nanomedicine could have on a tumor, there are several limitations to this method of nanoparticle administration. For example, high intratumoral pressure increases the likelihood that nanoparticles will leak out of the tumor if the injection is not done slowly. Additionally, they lack relevance for clinical translation since many tumors in human subjects are not accessible to intratumoral injection. Therefore, these types of injections should be used sparingly in the preclinical setting.

4.2. Experimental Control Groups in Nanomedicine Studies

When developing cancer nanomedicines, it is critical to include proper controls to ensure that the nanocarrier itself does not induce adverse effects and that tumor inhibition is derived from the intended mechanism of action. It is also important to eliminate as much human bias as possible.[48] When feasible, in vivo studies should be performed in a blinded manner, for example, by mixing treatment groups among various cages or having a researcher who is unaware of which treatment an animal received perform any measurements of tumor volume or assessment of histology slides. This subsection provides an overview of specific controls one might consider for various types of nanoparticles and tumor models. This list is not all-inclusive but is intended as an overview.

4.2.1. Controls to Account for Nanoparticle Mode of Action

Proper nanoparticle controls depend upon the mechanism through which the nanoparticle achieves its effect. As examples, controls for two major classes of nanomedicines are discussed: photoresponsive therapies and gene regulatory agents.

The two main classes of photoresponsive therapy are photothermal therapy and photodynamic therapy, in which administered nanoparticles are excited by externally applied light to induce cell death through the production of heat or reactive oxygen species, respectively.[49,50] These are considered precision therapies that enable spatiotemporal control, as the therapeutic effect should be achieved only when and where both the nanoparticles and light are applied. To ensure the nanoparticles and light are innocuous when administered individually, researchers should always include the following treatment groups in preclinical animal testing: saline (or other vehicle control), saline and applied light, nanoparticles alone, and nanoparticles with applied light (**Figure 4**A). If the phototherapy is working as anticipated, tumor inhibition should occur only for the combination of nanoparticles and light.

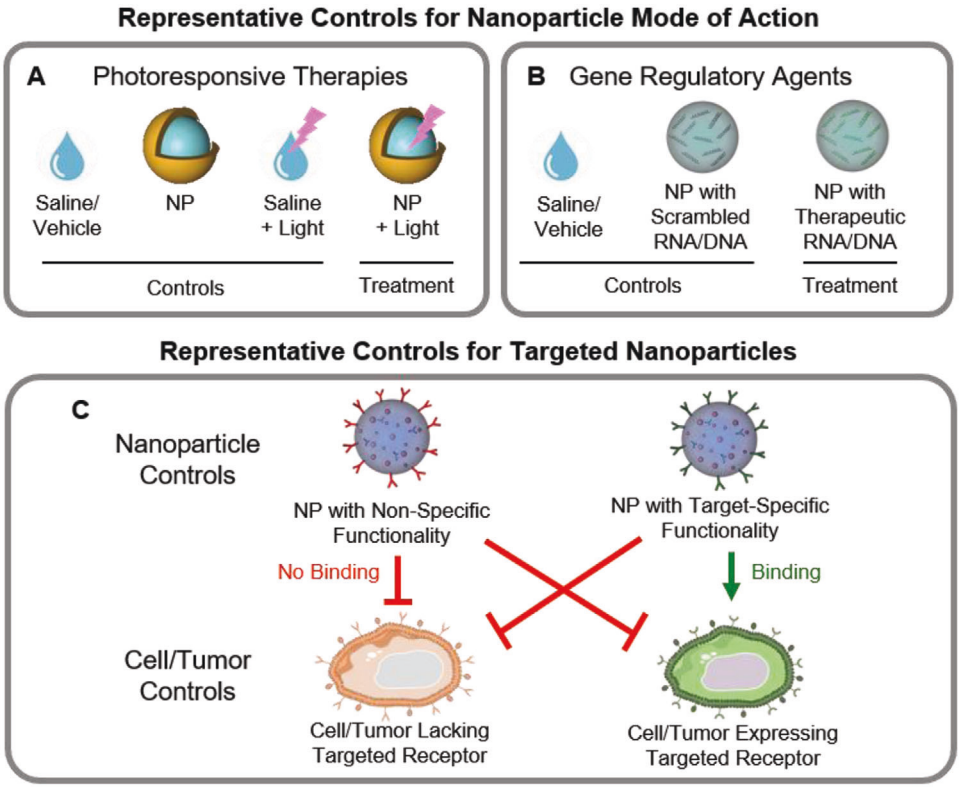

Figure 4. Overview of experimental control groups to include in studies of cancer nanomedicine. Researchers should include appropriate controls for nanoparticle mode of action and surface functionality in all studies. A) Scheme depicting appropriate controls to include when evaluating photoresponsive therapeutics. B) Scheme depicting proper controls to include when evaluating gene regulatory agents. C) To evaluate targeted nanomedicines, researchers should include both nanoparticle controls and tumor controls in their study design to validate the specificity and efficiency of target-specific binding. Portions of this figure were produced with permission using Servier Medical ART templates, which are licensed under a Creative Commons Attribution 3.0 Unported License from Servier Medical Art; https://smart.servier.com.

As a second example, many nanoparticles are designed to facilitate gene regulation and control tumor growth through the intracellular delivery of nucleic acids such as RNA and DNA. There is evidence in the literature that simply introducing exogenous nucleic acids into cells can change cellular gene expression and thus influence cell fate.[51] This potentially results from saturation of and competition for the RNA-induced silencing complex (RISC). When evaluating gene regulatory nanomedicines, it is therefore important to investigate the effects of nanoparticles carrying both the target-specific RNA/DNA as well as nanoparticles carrying a nonsilencing scrambled sequence of the therapeutic RNA/DNA (Figure 4B). This will ensure that any therapeutic effects observed are due to the specific nucleic acid delivered and not due to other factors.

4.2.2. Controls to Account for Nanoparticle Surface Functionality

Many cancer nanomedicines incorporate targeting agents to facilitate cancer cell-specific binding and delivery of their cargo. Targeted nanoparticles can be broadly classified in two categories: bioconjugated systems and biomimetic systems.[52] Bioconjugated nanoparticles have antibodies, peptides, or other small molecules conjugated to their surface to facilitate binding with specific receptors found on the surface of the target cells. To evaluate whether the targeting agent is effective, researchers should include as an experimental group either uncoated nanoparticles or nanoparticles that have a nontargeting antibody/peptide/small molecule on their surface (Figure 4C). A similar concept holds true for biomimetic nanomedicines, which typically consist of nanoparticles wrapped with cell-derived membranes.[52,53] Previous studies have shown that cancer cell membrane-coated nanoparticles can accumulate in tumors that are matched to the cell source; for example, breast cancer cell membrane-wrapped nanoparticles will preferentially enter breast cancer tumors versus other tumor types in mice.[54,55] This is known as homotypic targeting. When evaluating these systems, researchers should compare nanoparticles wrapped with the target-specific membranes (e.g., breast cancer cell membranes) with unwrapped nanoparticles and/or nanoparticles wrapped with nontargeted membranes (e.g., those derived from red blood cells or another cancer type). This will confirm whether homotypic targeting is occurring as anticipated.

Beyond including nanoparticle controls as described above in the study of targeted nanomedicines, researchers should also include tumor controls. Specifically, researchers should administer nanoparticles to mice harboring tumors with high expression of the targeted receptor or with low or absent expression of the targeted receptor (Figure 4C). This is important to elucidate the

effect of receptor expression on nanoparticle accumulation and validate the targeting efficiency.

4.2.3. Tumor Inoculation Controls

As a final consideration, beyond controlling for nanoparticle type and tumor type, researchers may need to account for the tumor inoculation procedure itself. The procedure used to implant cancer cells may disrupt tissue, leading to altered nanoparticle accumulation. For example, a major challenge associated with the treatment of brain tumors is crossing the blood–brain barrier (BBB).[56] When cancer cells are injected intracranially to initiate brain tumor growth, this can disrupt the BBB and potentially alter the accumulation of nanoparticles within the area.[57] By injecting saline (or the medium in which the cells are suspended) in the same manner as the tumor cells, researchers can assess whether any accumulation of nanoparticles within the tumor is due to the properties of the nanoparticle and/or the effect of the tumor on the tissue environment.

To summarize, Sections 3 and 4 have emphasized various factors researchers must consider when designing animal studies. In Section 5, animal welfare considerations are presented, which must be a top priority for all nanomedicine researchers.

5. Animal Welfare

Animal welfare considerations are of the utmost importance during the preclinical testing of cancer nanomedicines. The American Association for Laboratory Animal Science (AALAS) works to advance responsible laboratory animal care and use, offering educational materials and certification programs for laboratory animal researchers. Every institution using animals for federally funded research must have an AALAS approved and managed Institutional Animal Care and Use Committee (IACUC), which is responsible for administering and reviewing animal use protocols (AUPs), providing training for proper animal care and use, and conducting evaluations of the institution's animal care.[58] The guidelines for animal welfare discussed in this section are based on typical IACUC recommendations nationwide.

5.1. IACUC Animal Use Protocols

To begin legal animal work at an institution, an AUP must first be filed with the local IACUC. **Table 1** provides a checklist to help researchers establish an AUP and a checklist to ensure animal welfare throughout protocol execution. An important component of the AUP is the reasoning for the number of animals to be used in the study. The group size should be determined statistically with power analysis (preferred method) or based on the number of cells or tissues required for planned ex vivo work. If too few animals are used, then a significant result may be missed. If too many animals are used, then valuable resources are wasted. Thus, it is imperative to statistically determine the appropriate sample size in advance of animal studies. Researchers reading this article are assumed to be familiar with power analysis but should turn to the Handbook of Biological Statistics[59] or another statistics textbook if needed.

Table 1. Checklist for establishing an animal use protocol and maintaining animal welfare throughout protocols.

Checklist for establishing animal use protocols
Contact institution's IACUC and complete necessary training modules
Write animal use protocol (AUP) and gain IACUC approval. Include the following components in AUP:
Nonscientific summary of scientific goals and significance of study
Detailed experimental design, including:
Species identification
Appropriateness of species and numbers used
Justification for use of animals over in vitro methods
Statistical justification for the requested number of animals
Description of proposed use of animals
Detailed description of planned procedures
Description of criteria and methods for euthanasia

Checklist for maintaining animal welfare throughout protocols
Perform studies, consulting with veterinary staff as needed to ensure animal welfare. Throughout experiments, monitor animals for:
Tumor size (maintain diameter below 1.2 cm for subcutaneous tumors in mice)
Weight (ensure weight loss does not exceed 20%)
Aggressive behavior (separate animals into distinct cages if needed)
Signs of distress (hunched posture, difficulty ambulating, ruffled fur, labored breathing, abdominal distension, persistent hypothermia, incontinence or diarrhea, discharge of blood, mucus, or pus)

5.2. Minimizing Pain and Distress during Mouse Injections

The selected method for administering a nanomedicine (see Section 4.1) must be performed in a way to minimize unnecessary discomfort to the animal. **Table 2** provides a checklist to guide researchers when performing animal injections. Considerations for each type of injection are provided. For example, for tail vein injections, an IACUC-approved heat source, such as warm water or a heat lamp, should be used to dilate the veins and ease visualization. The animal should also be restrained in a special device, such as a plexiglass staged cone, to allow the tail to be pulled through one end for visualizing the vein while preventing the mouse from turning to bite or scratch the researcher.

5.3. Using Anesthesia

When defining an anesthetic regimen for procedures requiring anesthesia (tumor inoculation surgery, retro-orbital injections, laser irradiation,[60] etc.), researchers should consider the species and strain of the animal, its health status, safety measures, duration of the procedure, recovery time, and research goals.[61] **Table 3** provides a checklist for procedures involving anesthesia, including preprocedure planning as well as animal care during and after anesthesia. Anesthesia can be induced and

Table 2. Checklist for performing injections in animals.

Checklist for performing animal injections
Select injection type that is relevant to study and minimizes animal stress (choose one of the following)
Intramuscular (use caution to avoid sciatic nerve)
Intraperitoneal (dilute material with saline if needed to minimize irritation and increase absorption)
Retro-orbital (inject <200 µL per eye per day; apply ophthalmic ointment and allow to sit for at least 1 min; anesthesia is required)
Intratumoral (perform injection slowly to minimize leakage due to intratumoral pressure)
Subcutaneous (choose site between shoulders, in flank, or in inguinal region)
Intravenous (warm animal with proper device to dilate veins and use restraining device appropriate for species)
Define appropriate conditions for chosen injection type
Needle gauge
Syringe size
Needle length
Max volume of solution allowed (per injection and per day)
Volume of solution to be injected (must be less than maximum volume allowed)
Concentration of solution to be injected (ensure not too viscous)
Anesthesia method (gaseous or injectable) and length of anesthesia, if needed
Method for animal warming, if any (use caution not to burn or overheat animal)
Type and size of restraint device (sterilize device before use)
Other recommendations for animal injections
Before any injection, ensure needle and syringe do not contain air bubbles.
Inject needle with bevel pointing up.
Use fresh sterile needles and syringes for each mouse to minimize cross-contamination of experimental groups and prevent spread of disease.
Use minimum number of injections or injection attempts. If a tail vein injection must be reattempted, reinsert needle slightly above the prior injection site.
For intramuscular and intraperitoneal injections, perform aspiration to confirm blood vessels and nontarget tissues have not been pierced. If fluid is aspirated, remove and discard needle. Never inject aspirate back into animal.
Do not perform aspiration for intravenous injections, as this could collapse the vein.
After successful injection, apply pressure to the injection site with a sterile cloth or gloved finger for ≈1 min.
After injection, monitor animal for bleeding or signs of pain and distress. Treat as needed to resolve any issues before returning to cage.

Table 3. Checklist for procedures involving anesthesia.

Checklist for procedures involving anesthesia
Define methods in advance of procedure
Select anesthesia method (gaseous or injectable).
Define length of anesthesia.
Choose methods for warming animal and maintaining eye moisture if anesthesia will extend >5 min.
Animal care during procedure involving anesthesia
Induce anesthesia per selected method.
Warm animal as defined above, using caution to avoid burns or overheating.
Apply ophthalmic ointment or tape eyelids closed if procedure will be >5 min.
Perform pain stimuli tests to confirm induction. If animal retracts leg or vocalizes when pinched on tail or between toe webbing, the depth of anesthesia is insufficient.
Ensure proper level of anesthesia is maintained during the procedure. Stop anesthesia if animal exhibits irregular respiratory rate, rapid or slowed heart rate, or blue discoloration of visible mucosal membranes.
Animal care after anesthesia and procedures are complete
Move animal to dry and warm area for recovery. Recovery area should be free of wood or sawdust shavings.
Monitor animal until awake and stably moving.
Move animal back to routine housing with ambient temperature

maintained using gaseous or injectable methods. Gaseous anesthesia requires an induction chamber or delivery of anesthetic via a facemask or intubation with an endotracheal tube or nasal catheter. This method provides several advantages including increased control over depth of anesthesia, decreased cardiopulmonary depression, improved safety during induction, and reduced recovery time. By comparison, injectable anesthesia requires less equipment, less operator training, and lower initial costs.

If the animal is under anesthesia for more than 5 min, ophthalmic ointment must be applied to the animal's corneas or the eyelids taped shut to prevent damage to the cornea. Additionally, while under anesthesia, an external heat source must be used, such as a feedback-controlled, infrared, warm water, or air circulating device. Electric heating pads and heat lamps should not be the first choice due to their potential to cause burns during extended use. Selecting the correct external heat source is critical for rodent welfare during anesthesia-related procedures because hypothermia is a common cause of mortality in rodents due to their high surface area to body mass ratio.[62]

Once anesthesia is administered, pain stimuli tests must be performed to ensure the animal is fully induced and their perception of pain is blocked (Table 3).[62] If the depth of anesthesia is sufficient, the planned procedures may commence. During the procedure, the animal must be monitored to ensure the proper level of anesthesia is maintained. After completing the procedure, the animal should be moved to a warm and dry area for recovery. Adult animals should be kept at 27–30 °C and neonates at 35 °C during recovery.[62] Animals should be monitored until awake, stable, and walking normally, and then returned to their routine housing and ambient temperature.

5.4. General Considerations for Animal Welfare

After animal procedures are performed, various characteristics of the animal must be regularly monitored. These are summarized in Table 1 and reiterated here. Each animal's weight should be tracked throughout the tumor growth and treatment period, as mentioned before. The tumor length and width should also be measured regularly to calculate tumor volume over time and

monitor when euthanasia criteria have been met.[60] It is advised the same well-trained technician or researcher be involved for the duration of the study to perform measurements and animal care. This will reduce measurement variations and enable quicker notice of any adverse health effects or behavior.[60]

Therapies requiring laser irradiation may cause superficial reddening of the skin at the irradiation site. Restricting dosages and performing treatments no more than once daily should minimize this response. Skin peeling at the treatment site post irradiation should not be allowed to continue for more than 24 h. Modification of irradiation dosages should be explored if adverse skin side effects continue to occur and the animal care staff should be notified of these injuries. Tumor scabbing may occur in irradiated subcutaneous tumors and this can be a sign of response to treatment. However, if there is evidence of infection, lack of healing, or ulceration, the animal should be humanely euthanized.

5.5. Establishing Humane Endpoints

Humane endpoints should be established prior to beginning treatment to ensure animal welfare is upheld and adverse effects are minimized. Endpoints for particular cancer models should be cognizant of the known pathogenesis of the selected tumor model, such as rate of tumor growth and metastatic activity. Some example endpoints are summarized in Table 1. The site of solid tumors will influence the set endpoint for acceptable tumor volume. When tumors are formed in the footpad, tail, eye, bone, or intramuscular areas, special justifications and earlier endpoints are needed. Cancer models known to metastasize rapidly and/or to the brain also require justification and animal weight and health should be monitored frequently. For animals with one subcutaneous tumor, the mean diameter should not exceed 1.2 cm in mice or 2.5 cm in rats for therapeutic studies.[60] If two tumors must be grown simultaneously on the animal, the total size should not exceed the maximum burden of a single tumor.[60] Tumors must not be allowed to grow to a size that causes abnormal animal ambulation, vocalization, behavior, or function.

Besides tumor volume, other metrics can serve as humane endpoints. Weight loss greater than 20% at any time or 15% maintained for 72 h compared to pretreatment (day 0) weight of adult mice or age-matched vehicle controls should be a set endpoint for cancer studies.[60] If body weight is not heavily influenced in a particular cancer model, then muscle atrophy and emaciation or body condition scoring may be used to monitor animal health. Other characteristics that are study endpoints include persistent hypothermia, labored respiration, enlarged lymph nodes or spleen, or anemia (seen as pale feet). If an animal is suffering from significant abdominal distension with abdominal cavity fluid buildup greater than 10% of body weight or body girth increase of 20% of age-matched controls, this is an endpoint. Additional endpoints include indications of adverse side effects such as blood, mucus, or pus accumulation on bedding and incontinence or diarrhea over a 48 h period.[60]

Different countries regulate animal welfare during cancer research with contrasting limits of tumorigenesis and contradicting study endpoints. There should be a universal standard among nanomedicine researchers for animal welfare, especially concerning limiting the size of tumor growth. When study endpoints have been met, the animal must be euthanized with at least two IACUC-approved methods that are suitable for the intended downstream processing of excised tissues after the study. Intentionally using death as an endpoint is unacceptable and animals should never be allowed to become moribund.[60] Overall, treating animals with care and respect must be a top priority for all nanomedicine researchers.

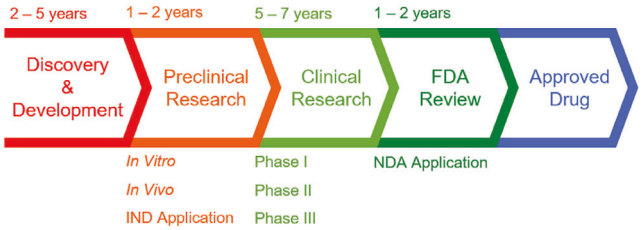

Figure 5. Scheme depicting the timeline from nanomedicine discovery to clinical implementation. Researchers evaluating cancer nanomedicines preclinically in vitro and in vivo must be cognizant of the requirements to initiate clinical trials and ultimately gain FDA approval.

6. Clinical Translation of Nanomedicines after Animal Studies

When designing studies to evaluate nanomedicines in vivo, researchers should consider the information that will need to be presented to the US Food and Drug Administration (FDA) to initiate clinical trials. The FDA has specific regulation pipelines in place that vary based on whether a system is evaluated as a drug, a device, or a combination product. Most nanomedicines are tested as drugs for human use, with evaluation and approval given by the Center for Drug Evaluation and Research (CDER).[63] The three phases of evaluation are summarized briefly in Section 6.1 (**Figure 5**). Then, Section 6.2 describes the current state of nanomedicines in clinical trials.

6.1. Phases of Evaluation toward FDA Approval

6.1.1. Preclinical Phase

The preclinical phase starts with the discovery of a potential therapeutic. This phase seeks to validate efficacy and assess toxicity in vitro and in vivo.[64] The Nanotechnology Characterization Laboratory (NCL) has a database of standardized protocols to evaluate new nanotherapeutics preclinically,[65] which researchers should use as a guide. In vitro studies strive to determine the nanomedicine's mechanism of action and evaluate the effects of any components of a nanotherapeutic against diverse cell lines. In vivo studies aim to elucidate any off-target effects and evaluate general safety. These studies typically include multiple orders of animal species, starting with mice and moving upward to species such as canines and nonhuman primates.[66] Preclinical data is compiled in an Investigational New Drug (IND) application that is filed with the FDA.[67] Submissions require data such as toxicity levels, manufacturing schemes, and clinical protocols. Researchers should be cognizant of these submission

Table 4. Nanoparticles in phase III and postmarketing phases of clinical development.

Phase III			
Identifier	Study title	Cancer	Status
NCT02123407	Clinical study on the harvesting lymph nodes with carbon nanoparticles for advanced gastric cancer	Gastric	Unknown
NCT00397761	Capecitabine and paclitaxel (albumin-stabilized nanoparticle formulation) in treating women undergoing surgery for stage II or stage III breast cancer	Breast	Unknown
NCT04033354	A randomized, double-blind, placebo controlled phase III study to investigate efficacy and safety of first-line treatment with HLX10 + chemotherapy (carboplatin-nanoparticle albumin bound (Nab) paclitaxel) in patients with stage IIIB/IIIC or IV NSCLC	Lung	Recruiting
NCT01583426	Nanoparticle-based paclitaxel versus solvent-based paclitaxel as part of neoadjuvant chemotherapy for early breast cancer (GeparSepto)	Breast	Unknown
NCT00785291	Paclitaxel, Nab-paclitaxel, or Ixabepilone with or without bevacizumab in treating patients with stage IIIC or stage IV breast cancer	Breast	Active, not recruiting
NCT00989131	Study of paclitaxel in patients with ovarian cancer	Ovarian	Completed
NCT00046527	Study of ABI-007 and Taxol in patients with metastatic breast cancer	Breast	Completed
NCT01644890	A phase III study of NK105 in patients with breast cancer	Breast	Completed
NCT04137653	Treatment of triple-negative breast cancer with albumin-bound paclitaxel as neoadjuvant therapy: a prospective RCT	Breast	Not yet recruiting
NCT03875092	A study of carboplatin-paclitaxel/Nab-paclitaxel chemotherapy with or without Pembrolizumab (MK-3475) in adults with first-line metastatic squamous nonsmall cell lung cancer (MK-3475-407/KEYNOTE-407)-China extension study	Lung	Active, not recruiting
NCT02775435	A study of carboplatin-paclitaxel/Nab-paclitaxel chemotherapy with or without Pembrolizumab (MK-3475) in adults with first-line metastatic squamous nonsmall cell lung cancer (MK-3475-407/KEYNOTE-407)	Lung	Active, not recruiting
NCT04060472	Paclitaxel (albumin-bound) and oxaliplatin for advanced hepatobiliary and malignant tumors	Hepatobiliary	Not yet recruiting
NCT04261777	Ferumoxtran-10-enhanced MRI in prostate cancer patients	Prostate	Recruiting
NCT03768414	Gemcitabine hydrochloride and cisplatin with or without Nab-paclitaxel in treating patients with newly diagnosed advanced biliary tract cancers	Biliary	Recruiting
NCT02379845	NBTXR3 crystalline nanoparticles and radiation therapy in treating and randomized patients in two arms with soft tissue sarcoma of the extremity and trunk wall	Soft tissue	Active, not recruiting
NCT04233866	Comparing two treatment combinations, gemcitabine and Nab-paclitaxel with 5-fluorouracil, leucovorin, and liposomal irinotecan for older patients with pancreatic cancer that has spread	Pancreatic	Not yet recruiting
Postmarketing phase			
Identifier	Study title	Cancer	Status
NCT03799679	Albumin-bound paclitaxel followed by epirubicin in combination with cyclophosphamide in triple negative breast cancer	Breast	Recruiting
NCT03799692	Albumin-bound paclitaxel combined with carboplatin as neoadjuvant chemotherapy in luminal B/HER-2 negative breast cancer	Breast	Recruiting
NCT00920023	Preoperative staging of pancreatic cancer using superparamagnetic iron oxide magnetic resonance imaging (SPIO MRI)	Pancreatic	Completed
NCT03401827	The effect of gemcitabine plus Nab-paclitaxel as secondary chemotherapy in advanced pancreatic cancer	Pancreatic	Unknown

requirements as they plan and perform studies to evaluate nanomedicines.

6.1.2. Clinical Phase

With IND approval, therapeutics next enter the clinical phase, which consists of human trials divided into another three phases (I, II, III) (Figure 5).[64] Phase I examines dosage levels, acute toxicity, and drug distribution in humans. Usually the drug (or nanomedicine) is first administered to healthy patients to set a baseline for preliminary dosing regimens. Phase II then evaluates efficacy in patients with the targeted disease. Last, phase III studies evaluate the nanomedicine in a large number of patients, who are randomized and include placebo controls.[68] If all three phases of human trials are successfully completed, a New Drug Application (NDA) can be filed with the FDA. If the NDA is approved, the nanomedicine can be marketed and distributed by medical practitioners.[69]

6.1.3. Postmarketing Phase

After NDA approval, the FDA performs continuous monitoring of new therapies in the postmarketing phase.[64] These studies aim to further validate efficacy and safety and can be undertaken

by the FDA, practicing clinicians, or manufacturers. If harmful effects appear that were not observed in clinical testing, the therapy may be removed from clinical use to ensure patient safety.

6.2. Current State of Nanomedicines in Clinical Translation

As of 2019, Clinicaltrials.gov lists 265 trials that are filtered for the condition "cancer" and the search term "nanoparticle."[70] The majority of these trials are in phase I or II evaluation, with 16 trials in phases III and 4 trials in the postmarketing phase (**Table 4**). This indicates that, although progress has been slower than researchers, clinicians, and patients desired, nanomedicine is still en route to having a substantial clinical impact. The FDA approved Abraxane, an albumin-bound paclitaxel nanoparticle, for the treatment of triple-negative breast cancer in 2005,[71] and Abraxane was also recently approved for use in combination with the immunotherapeutic Tecentriq.[72] Perhaps the most exciting development of 2019 was the FDA approval of Patisiran/ONPATTRO, a lipid-based siRNA delivery vehicle, for treatment of polyneuropathy caused by an illness called hereditary transthyretin-mediated amyloidosis.[73] Patisiran/ONPATTRO is the first FDA approved RNA interference therapy of any kind, and its landmark approval gives hope that other RNA delivery vehicles currently under investigation for cancer therapy will also be successful in clinical trials and beyond.

7. Conclusion

The current attrition rate of cancer nanomedicine in clinical trials highlights the importance of more effective preclinical evaluation. To maximize the success in transitioning nanomedicines from preclinical testing to human use, researchers should carefully design and execute their studies, and report the results with accuracy and transparency. Prior to animal studies, researchers should consider utilizing organ-on-a-chip or chorioallantoic membrane models to evaluate nanomedicines.[12] This might reveal which nanomedicines are most likely to succeed in rodent models and, ultimately, human subjects. Importantly, studies performed in lower order species such as mice should be constructed with transition to the next order in mind, and researchers should consider using models that account for factors such as the age of a typical cancer patient[74,75] and the role that inflammation plays in tumorigenesis.[76,77] Currently, most preclinical studies utilize young mice or rats, but cancer primarily affects older humans. Using older rodents for preclinical testing may improve the correlation between animal studies and clinical trial results. Besides these considerations, researchers should aim to involve clinicians at every stage of the design and development process as their experience and knowledge may facilitate the successful transition of nanomedicines to human use.

This report has outlined best practices for the preclinical evaluation of cancer nanomedicines, including the importance of animal welfare. At the experimental design level, researchers should use the most accurate tumor model for their particular cancer indication, as well as the most clinically relevant dosing route. It is important to include controls for nanoparticle design as well as tumor characteristics, as this will provide valuable information to inform further development. Further, it is imperative that researchers report their results with accuracy and without overstating the findings, as this will lead to the most rapid progress for the field as a whole. Ultimately, by implementing the guidelines provided here for thorough in vivo evaluation of cancer nanomedicines, researchers may improve the rate of nanomedicine translation into the clinic.

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Conflict of Interest

The authors declare no conflict of interest.

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