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Translational Therapeutics in Genetically Engineered Mouse Models of Cancer

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Advances in knowledge of the molecular alterations of human cancers, refinements in technologies for the generation of genetically engineered mouse models (GEMMs), and the development of cancer therapies have accelerated in recent years. Progress in these fields provides the foundation for clinically relevant studies to be performed in GEMMs, through which it is possible to glean information on drug efficacy and to identify determinants of sensitivity and resistance to drugs and drug combinations. GEMMs used in pre-, co-, and postclinical studies must closely recapitulate the genetics, histopathology, and response to therapy of the human disease. Prevention and intervention trials can be designed in GEMMs to test the effects of drugs on tumor initiation, regression, and progression. Given their complexity, careful consideration of the infrastructure requirements and practical aspects of each individual experiment, including enrollment, tumor monitoring, and dose and schedule, must be considered in the design of therapeutic studies in GEMMs. Advantages of GEMMs include the ability to rapidly perform drug efficacy studies in a defined genetic background, the ease of pharmacodynamic and pharmacokinetic assessments, and the possibility of experimentally manipulating model systems to address questions that cannot be addressed in patients. In light of these features, GEMMs are useful tools for translational studies to inform clinical trials in cancer patients.

GOALS AND USES OF THERAPEUTIC STUDIES IN MICE

Translational research stands at the interface of basic science and clinical medicine and can advance the aims of both disciplines. In this regard, precisely targeted pharmacological agents can be powerful tools for exploration of the fundamental biology of tumors, provided that the drugs are well understood and the studies well controlled. For example, as there are often significant differences between deleting a gene and inhibiting the activity of a protein (Kwong et al. 2012), paired studies using genetic ablation and pharmacological inhibition can provide both confirmation and context to the understanding of gene function. Furthermore, genetic strategies for acutely abrogating gene function in vivo following the development of a spontaneous tumor, although powerful, can be complicated and time-consuming. In contrast to most genetic studies, pharmacological studies proceed more rapidly once an appropriate infrastructure is established. More generally, pharmacological agents can be used for hypothesis testing and exploration of mechanisms underlying observed phenotypes.

At the other end of the spectrum, preclinical trials are designed to inform the decision on whether a drug should progress to human trials by evaluating the potential efficacy of the agent against specific

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types of cancer. The design, implementation, and interpretation of such experiments are each critical to their predictive utility (see below). However, once the clinical evaluation of a therapy is initiated, the role for translational therapeutics in mouse models does not end. Coclinical trials, performed side-by-side with human clinical studies, enable better interpretation of clinical trial data and offer the potential for the improved design of drug scheduling and doses, real-time development of pharmacodynamic assays, and rapid testing of novel combinations of drugs (Nardella et al. 2011). Finally, postclinical trials may be performed to better understand human clinical trial outcomes. In the instance of a positive outcome, this provides the opportunity to understand the mechanisms of response. In cases where tumors acquire resistance to therapy, this can be studied in the mouse and compared to human clinical samples. For trials in which a subset of patients respond to a therapy whereas others are innately resistant, mouse models can be used to explore the determinants of drug sensitivity. Finally, for clinical trials with a negative outcome, follow-up studies in mouse models are critical for understanding the discordance between preclinical and clinical results. This may shed light on how the drug may be better used or, conversely, how the mouse model failed to predict the actual outcome. Both end points are critical to future studies.

Standing in between these basic and clinical applications, translational studies can also help to understand the complex and dynamic biology that occurs within tumors following pharmacological perturbation. Homeostatic mechanisms and complex feedback loops can yield unanticipated effects when apparently linear pathways are altered through drug treatment. For example, inhibition of the mTORC1 complex by rapamycin and related compounds results in the compensatory activation of an upstream component of the pathway AKT (Sun et al. 2005). By studying these mechanisms, new combination approaches may be developed with potent efficacy against otherwise recalcitrant cancers.

FEATURES OF A TRANSLATIONAL MOUSE MODEL

Every model system has its strengths and weaknesses. The key to selecting the right model system is to focus on the goals of the research. When asking a basic question about whether a given gene or pathway can play a role in a specific function, simple models that are quick and easy to use may be appropriate. But for the purpose of determining whether a drug should be tested in patients, it is critical that the best possible models be used for the preclinical studies. Similarly, coclinical and postclinical studies are critically dependent on the predictive accuracy of the model system used, as well as the ability to recreate effects that have been observed in patients. Several characteristics should be considered in making this determination.

First, the model should have similar genetics to the human disease. For some cancers, this can be straightforward: Pancreatic ductal adenocarcinoma has just four high penetrance genetic alterations that characterize the vast majority of all such tumors. In other cases, such as breast cancer, there are few archetypal mutations that characterize the disease; several molecular subtypes have been identified within breast cancer, each of which has different mutation patterns. In this case, care should be taken to identify the most relevant subtype for the work or to use a variety of different models to represent the diversity of the human disease.

A second feature to be considered in choosing a disease model is its pathological fidelity, including the histopathological features of the tumor, aspects of its progression through invasion and metastasis, and the nonneoplastic comorbidities that arise during the course of the disease. For example, a number of GEMMs have been engineered that develop metastases, sometimes even in the appropriate anatomical sites relative to the human disease. However, not all mouse models actually die of metastatic disease burden; many succumb to locally destructive disease at the primary site. Most cancer patients ultimately succumb to the burden of disseminated disease or comorbidities such as infection or cachexia. These distinctions should be considered when selecting a model system, when interpreting the results of preclinical studies, and ultimately during the design of clinical trials arising from successful preclinical experiments.

Third, attention should be paid to the acquired genetic alterations that arise during the natural history of tumor development and whether these are similar to those found in the human disease. These may include established immunohistochemical markers, epigenetic changes, or genetic mutations and alterations that are known to arise in human tumors. Differences between the mouse and human genomes (both in terms of sequence and chromosomal structure) have the potential to affect the course of tumor progression. For example, syntenic regions that are easily codeleted in a single mutational event in one species may require two independent mutations in another species where they are located on different chromosomes (McClatchey et al. 1998). However, the overall changes to cancer signaling pathways may be preserved (Sweet-Cordero et al. 2005; Herschkowitz et al. 2007). Thus, it is worthwhile to evaluate genetic and epigenetic changes on a genome-wide scale to map alterations at the pathway level for comparison to the human disease.

Finally, the single most important feature of a high-quality preclinical tumor model is that it responds to existing drugs in a manner that faithfully recapitulates the human disease. This includes both drugs that are known to be effective (if available) and those known to lack efficacy (Olive and Tuveson 2006). Although often considered a "control" experiment, such "credentialing" studies are critical for establishing the predictive utility of a model system (i.e., its ability to predict the future success of novel agents when translated to a clinical setting).

THERAPEUTIC TRIAL STRUCTURES

Two principal trial enrollment structures may be used in therapeutic studies, depending on the biological question that is being addressed: prevention trials or intervention trials. Prevention trials address whether a drug affects tumor initiation or can prevent progression from premalignancy to invasive disease. Intervention trials, on the other hand, address the effect of a therapy on the maintenance or progression of established tumors. Prevention and intervention trials therefore have different criteria with respect to the stage of disease at the point of enrollment. Irrespective of the design of the trial, careful consideration must be given to animal enrollment, dose and schedule of treatment, and duration of treatment and tumor monitoring, as described in the Practical Considerations section of this article.

The conclusion of a trial may also vary based on the goals of the study. In a "survival study," physical or behavioral criteria are used as a surrogate for the overall survival of the animal. The actual death of the animal is not used as an end point, both for ethical reasons and because it is preferable to be able to acquire fresh necropsy tissues. It is important to establish a priori the exact survival criteria that will be used to decide when to euthanize the animal. Alternatively, in a "time point study," the trial can be terminated at a defined time after the drug was first administered. This structure can facilitate the analysis of rate of progression by comparing the extent of tumor growth between two groups within a defined period of time. Finally, a specific phenotype can be used to define the study end point (e.g., tumor formation, appearance of metastases, or measurable tumor regression). This latter "phenotypic end point study" is especially useful when tumors can be palpated, identified visually, or detected by imaging.

Prevention Trials

In a prevention trial, mice are enrolled before the development of tumors or when premalignant lesions are present. Ideally, therefore, a specific time point, after birth or after induction of the neoplastic lesion, is selected to initiate treatment before malignant tumors are observed. Imaging can be used to identify (or exclude) the presence of lesions before starting treatment. If imaging is not possible, the histopathology of the organ of interest in the model being analyzed must be well established at the specific time point in which drug treatment is initiated.

Heterogeneity with regard to tumor progression must be taken into consideration when designing these studies. For example, if only a very small fraction (<1%) of animals is predicted to have invasive

cancer at the beginning of the study, such animals are unlikely to influence the study outcome. However, if a significant fraction of animals (>5%) show invasive disease at study initiation, this could influence the outcome of the study and must be examined as a confounding variable. In a prevention study, the drug can be administered long term to determine whether constant administration of the agent is required to prevent tumor formation or progression to malignancy, thus impacting survival of the animal (Prevention Survival Study). This strategy is especially amenable to testing of drugs that are unlikely to have major side effects (i.e., vitamins) over a prolonged course of treatment. Agents can also be administered for a short period of time followed by assessment of the difference in survival or tumor initiation/progression between the control and experimental groups.

Tumor-prone GEMMs can be especially valuable for identifying drugs that can prevent progression to cancer (Grippo and Tuveson 2010). The recalcitrance of many advanced cancers has led to a strategy of aggressive intervention for many premalignant conditions, despite a paucity of definitive data demonstrating that this is always necessary. For example, patients diagnosed with ductal carcinoma in situ of the breast undergo treatment (surgery and in some cases radiation therapy) to prevent the disease from progressing to invasive cancer in a small fraction of total cases. Drugs that are effective at halting this progression could spare many women surgery. Moreover, for people with hereditary cancer syndromes, drugs that could decrease their risk of developing cancer would clearly be beneficial. One example of such a study used a mouse model of bladder cancer induced by loss of the tumor suppressor genes *p53* and *Pten*. In these mice, treatment of carcinoma in situ lesions with rapamycin prevented the development of invasive bladder carcinomas (Seager et al. 2009). These data point to a possible strategy to prevent progression in patients with bladder carcinoma in situ. Similarly, in a mouse model of *Brca1/p53* mutant breast cancer, treatment of the mice starting at 12 wk of age with the synthetic triterpenoid 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO-Me) delayed tumor onset and prolonged survival (Kim et al. 2012) by inhibiting the activity of Erbb2 and blocking proliferation. As illustrated by this latter example, experiments in GEMMs can provide insight into the efficacy and mechanism of drug action.

Intervention Trials

For patients diagnosed with advanced cancer, hope rests on the development of drugs that will halt or destroy an established tumor. In intervention studies, drugs are administered to animals that already have invasive cancer to determine whether treatment with a given agent reduces tumor burden, slows tumor growth, and/or has an impact on survival. Intervention studies can be performed in mice with chemonaïve tumors or in the refractory setting after tumors develop resistance to an initial agent. As with prevention trials, one must define the end point criteria to fit the goal of the study ("survival," "time point," or "phenotypic"). Of paramount importance to intervention studies are accurate imaging or tumor size measurements, which are required as readouts of therapeutic efficacy. Moreover, an appropriate length of drug-treatment must be selected to draw conclusions regarding drug efficacy, especially for time-point design studies (see Interpretation of Trial Outcomes).

INTERPRETATION OF TRIAL OUTCOMES

The ultimate goal of translational therapeutic studies is to accurately predict, as well as interpret, the effects of a treatment in patients. The performance of an agent in the clinic serves as a gold standard to which translational studies will eventually be compared. Therefore, a measured interpretation of translational data that factors in the subtleties of the model system, the pharmacology of the therapeutic agent, and the many differences between mice and humans should be undertaken. The finding of a minor, but statistically significant, difference in survival between treatment groups should be treated with caution and subjected to careful analysis of the context. A number of questions should be considered.

1. Did imaging studies show true tumor regression or only a decrease in tumor growth rate? Although slow tumor growth will lead to increased survival in a mouse model, it is considered tumor progression in a clinical trial, and would lead to a negative outcome. Furthermore, the growth rate of mouse tumors can be much higher than the corresponding human tumor, potentially enhancing the effects of drugs that target proliferating cells.

2. If regression or disease stabilization were noted, was the effect transient or prolonged? Whether a brief, minor regression represents bona fide tumor regression or anti-inflammatory effects on the tumor stroma (for example) depends on the drug and model system being used. However, premature termination of a study could lead to the incorrect assumption that a drug is effective (or ineffective). In a clinical setting, imaging is typically performed every few months, so a transient effect may never be detected and would likely have a negligible impact on overall survival. Immunohistochemical analysis of treated tissues at the time of regression can help identify the nature and cell-type specificity of drug effects.

3. Did a small subset of animals have substantial responses whereas the remaining tumor-bearing animals were insensitive to treatment? A large effect on a small fraction of patients can be challenging to distinguish in a clinical trial unless a biomarker can be identified that can distinguish sensitive from resistant tumors a priori. However, the discovery of such a biomarker could be profoundly relevant for patients, enabling an effective treatment for those most likely to benefit, while saving others from receiving ineffective treatment. Thus, the detailed investigation of rare animals that respond may ultimately prove rewarding.

4. What was the pathological context of the increased survival with respect to disease stage? Effects in a model in which death is due to locally destructive disease may not translate to a clinical trial conducted in a metastatic setting. Moreover, a careful assessment of the cause of death in each animal, and of tumor response to treatment, can help distinguish drugs whose efficacy is due to biological effects on the tumor from those that prolong survival for other reasons.

PRACTICAL CONSIDERATIONS IN THE DESIGN OF THERAPEUTIC STUDIES IN GEMMs

Enrollment Criteria

Crucial to the accuracy of data collected from intervention trials is the enrollment of animals with a similar tumor burden and with tumors at similar stages of progression. In models in which tumors are visible without imaging (e.g., breast cancer and skin cancer models), mice can be enrolled in the study based on a predetermined size of the lesion. In models in which the tumors arise internally, accurate imaging technologies that allow quantification of the size of the lesions should be established such that each mouse can be imaged and the tumor volume determined before enrollment. Imaging or tumor size–based enrollment in an intervention trial is preferable to time-point-based enrollment. The latter design assumes that individual mice will each have tumors of similar size at a given time point. Although time-point-based enrollment can be used for certain mouse models where tumorigenesis is exceptionally homogenous, this method is not ideal for most GEMMs.

Dose and Schedule

The second element that must be considered when designing a trial is to select an appropriate dosing and schedule regimen for the mice. This requires pilot experiments to determine the concentration and schedule of drug required to effectively hit the target. Ideally, a dose and schedule that allow for constant target inhibition, yet can be tolerated by the animal, are chosen. Target inhibition can be determined by treating mice with varying doses of drug and sacrificing the mice at specific time points after treatment. The range of doses tested can be selected based on knowledge of the maximum tolerated dose (MTD) of the drug used in xenograft studies. Because the MTD in immunodeficient mice, however, does not always correlate with the MTD in immunocompetent mice, it is important to test a range of doses in GEMMs. The dose and schedule are then determined by identifying the lowest

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dose that inhibits the target in the tumor for the longest amount of time to minimize dosing frequency. As an example, the MTD for the tyrosine kinase inhibitor erlotinib is 100 mg/kg/d in xenografts (Higgins et al. 2004). However, at 25 mg/kg/d, inhibition of the kinase activity of its target, epidermal growth factor receptor (EGFR), is achieved and is sustained for 24 h. Therefore, mice can be treated once daily at 25 mg/kg/d with this drug (Politi et al. 2006; Gong et al. 2007). Finally, drugs that require daily dosing pose a logistical challenge because this requires personnel available for drug treatments on weekends and holidays, especially for long-term treatments. It is important to consider this when designing studies and, when possible, devise a schedule that is biologically rigorous but also logistically feasible.

Length of Treatment

The duration of treatment chosen for the study is likely to be based on availability and tolerability of the drug. Furthermore, the nature of the drug under investigation may play a role in choosing the length of treatment. For example, tyrosine kinase inhibitors are taken daily by patients without interruption whereas many standard chemotherapeutics/biologics are given for a defined amount of time. Trial design in mice should take into consideration how the drugs are likely to be administered to patients.

Routes of Drug Delivery

There are several ways of administering drugs to mice and multiple factors that are considered when selecting the optimal route for each individual drug and study design. A detailed description of the routes of administration of drugs to mice is beyond the scope of this article and the readers are referred to excellent publications covering this topic (Turner et al. 2011a,b). Here we provide an overview of considerations to be made when selecting the route of drug delivery.

1. Is local or systemic delivery required? For example, skin lesions may only require topical administration of a drug to test the drug's efficacy. In contrast, even systemic administration may be inadequate for drugs whose target is in the central nervous system if they fail to cross the blood–brain barrier.
2. If systemic delivery is required, is the drug currently in use in patients? Because the route of delivery influences the pharmacokinetics (PK) and pharmacodynamics (PD) of the drug, it is best to use the same route used in patients.

The most common methods of systemic drug delivery used in mice are intraperitoneal (i.p.) or intravenous (i.v.) injection and oral/orogastric gavage. i.p. injection is used frequently in rodents in lieu of i.v. injection, since larger volumes of drug can be administered i.p. in mice and the method is less technically challenging. i.p. and i.v. deliveries are preferable for drugs that are poorly absorbed or degraded by the digestive system, such as antibodies. Oral delivery (p.o.), typically by oral gavage, allows the precise administration of a drug to the animal, rather than placing the drug in food or water. This technique, which requires trained individuals, can be used to rapidly dose large numbers of animals without requiring sedation. Oral gavage is used to deliver drugs that are absorbed well by gastrointestinal tract and are not adversely affected by hepatic metabolism. Because this is the most common mode of drug delivery in humans owing to its convenience and low cost, many drugs are administered via this route.

Tumor Monitoring

Tumor burden must be monitored regularly throughout and following treatment either by direct measurements or imaging, as appropriate for individual tumor models. The frequency with which tumors are monitored depends on the rate of growth of the tumors in the absence of drug and the feasibility of imaging or performing tumor measurements. As a general rule, at a minimum the tumor burden should be assessed before treatment and at the end of treatment or end of each treatment cycle.

TECHNIQUES USED IN THERAPEUTICS STUDIES

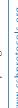### Pharmacodynamic and Pharmacokinetic Measurements

One of the most challenging goals in translational therapeutics is to identify the molecular mechanism underlying an observed phenotype. To begin to understand the interplay between therapy and response, two broad categories of pharmacological studies may be used: PK and PD. Put simply, PK looks at what the body does to a drug, whereas PD looks at what a drug does to the body. PK experiments are designed to measure how a drug is absorbed into the body, where and how quickly it is distributed throughout the body, the sequence of steps that occurs during drug metabolism as well as their compartmentalization, and, finally, how the products of metabolism are eliminated from the body. Together, these data paint a detailed picture of the life history of a drug as it passes through the body and provide critical insights into how best to use that drug. For example, one can use PK experiments to determine the effective concentration of drug achieved in different tissues, which may explain the sensitivity of different organs or tumors to treatment. Understanding the activating and inactivating metabolic pathways of a drug may provide insights into how to augment its efficacy or lessen toxicities.

An interesting case study is that of gemcitabine, a nucleoside analog that has served as the standard treatment for pancreatic cancer since 1997 (Burris et al. 1997). A circulating enzyme, cytidine deaminase, is responsible for the rapid deamination of the parent drug, leading to a short half-life of just 8 min (Abbruzzese et al. 1991). Furthermore, the parent compound is a prodrug that must be actively imported into cells and sequentially phosphorylated to create the active metabolite, gemcitabine triphosphate (Bergman et al. 2002). PK studies in multiple organ systems have showed that it is the concentration of the active metabolite in tumors that is the most accurate predictor of gemcitabine efficacy. This understanding has led to numerous efforts to improve the delivery of gemcitabine to tumors and to alter its metabolism, with several approaches having now progressed to clinical trials (Olive et al. 2009; Jacobetz et al. 2012; Provenzano et al. 2012).

PD experiments focus on the biochemical effect of the drug on its target and physiological consequences on the body. The breadth of studies that might be considered for PD analysis is much greater than for PK, encompassing all of the biochemical and molecular biology techniques that might be used to understand how a biological system has been perturbed. PD studies can be broken down by their proximity to the immediate activity of the drug. For example, direct PD experiments might study the phosphorylation state of a residue that is a kinase substrate following treatment with a targeted kinase inhibitor. In contrast, indirect PD experiments might measure the downstream effectors that are normally activated by the kinase. This can extend to more general cellular measures of response to therapy, such as markers of proliferation rate, apoptosis, DNA damage, or autophagy (see Table 1).

TABLE 1. Common immunohistochemical markers used to assess therapy response

Cellular process	Marker	Antibody	Dilution	Antigen retrieval
Proliferation	Ki67	Novocastra NCL-L-Ki67-MM1	1:200	Boiling, citrate unmasking
	Phospho-histone H3	Cell Signaling Technology #9701	1:100	Boiling, citrate unmasking
Apoptosis	Cleaved caspase	Cell Signaling Technology #9661	1:100	Boiling, citrate unmasking
	TUNEL	Roche, In Situ Cell Death Detection Kit #11684817910	Per protocol	Proteinase K provided with the kit
DNA damage	γH2AX	Cell Signaling Technology #2577	1:100	Boiling in 1 mM EDTA, pH 8, pressure cooker

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GEMMs are powerful tools for PK/PD studies because precise measurements of drug levels and biochemical markers can be made at multiple time points and dose levels, and samples can be more readily extracted from a broad range of tissues and fluids. Often, PK measurements require specialized analytical techniques such as liquid chromatography and mass spectrometry (LC/MS); it may be advisable to make arrangements with a pharmacology laboratory to perform the analysis of drug levels from snap-frozen tumor samples. Assays used in PD studies are more familiar to a molecular biology laboratory, including immunohistochemistry, immunoblotting, enzyme activity assays, enzyme-linked immunoabsorbent assays (ELISAs), quantitative reverse transcription polymerase chain reaction (RT–PCR), and other molecular techniques. In each case, careful consideration should be paid to the timing with which samples are collected relative to final dose administered to the animal. If the condition of the animal allows it, a final dose should be administered at a predetermined time point before necropsy for all animals in the study to minimize variation.

Tumor Biopsies

Genetic and phenotypic heterogeneity are simultaneously an advantage and disadvantage in the context of therapeutics studies. On the one hand, human tumors are typically quite heterogeneous because of the genetic diversity of the human population and the long timescales over which human tumors evolve. The comparative homogeneity of some GEMMs developed on inbred backgrounds has been a critique of this approach to tumor modeling. However, some exceptions exist, particularly models that incorporate point-mutant alleles of *p53*, loss of telomerase function, or other alterations predisposing to genomic instability. Reflecting the heterogeneity of human tumors is desirable for improving the fidelity of the model. However, it introduces practical problems related to data analysis. Increased variance within groups necessitates larger sample sizes to confidently detect a given effect size. Thus, any experiment that compares independent groups of tumors from a heterogeneous model will require significant resources. An alternative approach is to acquire paired samples at different time points from individual tumors. This helps to address issues of intertumoral heterogeneity, although it does not address intratumoral heterogeneity.

Surgical approaches to sampling surface lesions, such as skin and mammary tumors, are straightforward. Biopsies from abdominal organs are also feasible in most cases, although the involvement of tumors with major blood vessels is a significant concern (see Protocol: **Acquisition of Mouse Tumor Biopsies through Abdominal Laparotomy** [Sastra and Olive 2014]). Tumors within the thoracic cavity or in the central nervous system are extremely challenging to sample, although efforts are underway in these areas too. Ultimately, the amount of biopsy material that can be retrieved will determine the type and number of assays that may be performed. This may range from a few cells in a fine-needle aspirate to up to 20 mg of tissue from a 3-mm core biopsy.

Several study designs are facilitated through the use of paired biopsies. Pre- and posttreatment tumor samples may be compared to study the mechanisms of response to therapy. Comparisons of pretreatment biopsies from tumors that subsequently prove to be resistant or sensitive to therapy may be used to identify determinants of primary drug sensitivity and resistance. Pre- or intertreatment specimens can be paired with biopsies of tumors that have escaped treatment to study mechanisms of acquired drug resistance. Together, these study structures add a dynamic set of tools to the repertoire of preclinical studies.

PRECLINICAL INFRASTRUCTURE

The typical academic laboratory structure is organized around the educational goals of the laboratory: Graduate students and postdoctoral fellows lead independent projects, sometimes supported by technical staff who excel at individual techniques or who assist with specific projects. However, translational therapeutics studies require an investment in infrastructure to best facilitate the diverse techniques necessary for their execution. These include dedicated personnel, dedicated procedure space within an animal facility, access to small animal imaging equipment and expertise, the

space and expertise to carry out surgical techniques, and expertise in drug administration and sample acquisition from living and necropsied mice. These components may be organized into a preclinical core facility, either on a small scale within an individual laboratory or centrally within an institution. In addition, arrangements must be made for access to services such as histology, pathology, and analytical pharmacology and must also include a plan for information management.

Executed within an individual laboratory, the construction of a preclinical mini-core results in a modified academic organizational structure in which individual graduate and postdoctoral scientists interact and partner with the scientists of the preclinical core. For example, if an intervention study requires mice to be aged and imaged until tumor development, this portion of the project may be carried on an ongoing basis by the preclinical core until they identify animals with enrollment-ready tumors. These animals are then handed off to the project leader, typically a student or postdoctoral researcher, who will work in concert with the core to carry out the remaining procedures. In this way, batches of mice are provided to individual projects in turn. This provides both an economy of scale for the laboratory and improved efficiency for the individual scientists who can focus their energies on other tasks when not enrolling mice.

The specific personnel within a preclinical core will vary based on need, but in the simplest version, a staff scientist manages the overall process and contributes to individual technical procedures such as imaging and surgery, whereas a junior technician attends to the day-to-day dosing, sampling, and necropsy procedures. It is critical that these scientists be backed up by other members of the group to account for vacations, illness, and other unanticipated disruptions. Therefore, all members of the laboratory should be familiar with the technical procedures, and an organizational plan should be implemented that makes it possible for anyone in the laboratory to determine which animals are being dosed with which drugs on a given day. Coverage on weekends and holidays is critical and must be arranged in advance.

The facility in which procedures take place need not be very large, but ideally will be dedicated solely for translational therapeutics work. There will be high-volume traffic in the procedure room with numerous animals to be dosed, imaged, and necropsied and to undergo surgery. Surgical procedures require dedicated induction, maintenance, and recovery areas, with appropriate warming and monitoring equipment at each. Because of the frequent utilization of this space on a daily basis, it is critical that the procedure room be located within the animal facility where the animals are housed. Consideration should be given to the direction of airflow within the room relative to the rest of the facility, as many procedures require animals to be removed from microisolator cages outside of a procedure hood. Particularly in cases where a specific-pathogen-free (SPF) barrier is maintained, careful advance preparation may be necessary to sterilize all equipment and supplies that are used in the procedure room.

Small animal imaging is typically provided through core facilities or shared resources. It is again important to ensure frequent and bidirectional access between holding and imaging facilities to enable longitudinal studies. Although numerous small animal imaging modalities are available, it is important to consider the impact that each modality may have on the outcome of a treatment study. For example, frequent micro-CT may expose an animal to "potentially therapeutic" levels of ionizing radiation whereas magnetic resonance imaging (MRI) often requires administration of contrast agents that can be toxic, particularly in animals with compromised liver or renal function secondary to their tumor burden. Frequency, duration, impact, and timing of imaging sessions must be factored into the overall schedule for the animals to avoid interactions with the absorption of drugs. For example, large volumes of injected saline are often used with abdominal ultrasound in mice. This will dilute or prevent the absorption of drugs injected intraperitoneally, and so should be scheduled on alternate days from, or later in the day after, a drug injection.

Finally, close attention must be paid to information management. The amount of data created from an individual animal during translational studies is large, and a high-volume core will produce far more animals than can be accommodated by paper-based records. Spreadsheet-based approaches are possible, but ultimately limiting. Ideally, a dedicated database approach can be implemented to manage such studies, effectively creating an "electronic medical records" system for mice.

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CONCLUSIONS: WHY SHOULD WE PERFORM CLINICAL TRIALS IN GEMMs?

Testing the Efficacy of New Therapeutic Agents for Cancer Treatment

Studies in GEMMs have led to the successful implementation of new therapeutic strategies for cancer treatment. For example, one of the first targeted agents, all-trans retinoic acid (ATRA), was found to lead to responses in acute promyelocytic leukemia (APL) through testing of the agent in mice harboring the characteristic PML-RARα translocation (Brown et al. 1997; Grisolano et al. 1997; He et al. 1997). Ultimately, this work led to the curative regimen of arsenic trioxide and retinoic acid used today for patients with APL.

Resistance to Cancer Therapies

GEMMs are being used more widely not only to test new targeted therapies, but also to understand and overcome mechanisms of resistance to these drugs (see Politi et al. 2010; Politi and Pao 2011; Protocol: **Generation of Drug-Resistant Tumors Using Intermittent Dosing of Tyrosine Kinase Inhibitors in Mouse** [Pirazzoli and Politi 2014]). For example, mutations in the epidermal growth factor receptor (EGFR) are found in 10%–15% of lung adenocarcinomas (a histological subtype of lung cancer) (Lynch et al. 2004; Paez et al. 2004; Pao et al. 2004). Tumors with EGFR mutations are sensitive to treatment with EGFR tyrosine kinase inhibitors (TKIs), like erlotinib, that are now routinely used in the clinic to treat patients with this disease. Although >70% of EGFR mutant lung adenocarcinomas respond to these drugs, drug resistance typically emerges within a year of treatment. In most cases, resistance is due to the emergence of a secondary mutation in EGFR—the T790M mutation (Pao et al. 2005). Mouse models of TKI-resistant disease harboring the original sensitivity-conferring mutation (L858R) and the T790M mutation were used to test a variety of second-generation TKIs, EGFR antibodies, and combinations of these drugs. In these experiments, the combination of afatinib, an irreversible EGFR TKI, and cetuximab, an antibody to EGFR, produced dramatic responses in these GEMMs (Regales et al. 2009). This drug combination was used to design a clinical trial for patients with TKI-resistant EGFR mutant lung cancer that is showing an unprecedented 40% response rate in this population (Janjigian et al. 2011). A phase III study of this drug combination in patients with EGFR mutant lung cancer resistant to EGFR TKIs is currently planned and studies are ongoing to evaluate the ability of this combination to delay the onset of resistance when used as first-line treatment.

Although targeted agents are becoming more common in the clinic, the vast majority of metastatic cancers are treated with cytotoxic chemotherapies, which are frequently only palliative solutions because of the emergence of resistance. GEMMs, which have defined genetic lesions, are ideal for studying mechanisms of resistance to chemotherapeutic agents within genotypic subgroups. In a mouse model of *Brca1/p53* mutant breast cancer, doxorubicin and docetaxel were shown to give rise to drug-resistant disease because of up-regulation of the drug transporters Mdr1a and Mdr1b (Rottenberg et al. 2007). Cisplatin, in contrast, led to sustained tumor regression, highlighting the potential superiority of this drug for the treatment of BRCA-deficient breast cancer.

The Tumor Microenvironment and Response to Drugs

Tumors are complex entities that are intimately associated with elements of the host organism. In addition to the neoplastic cells that classically define a malignancy, a remarkable diversity of cell types may be contained within a given tumor and play an important role in its biology. These include fibroblasts, endothelial cells, numerous types of leukocytes, and even neuronal cells. These cellular constituents also fabricate and organize the extracellular matrix environment within the tumor. One of the principle advantages of GEMMs is that they maintain an intact immune system, resulting in a

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TABLE 2. Select examples of therapeutic trials in GEMMs

Study design	Cancer type	Reference(s)
Preclinical	Lung	Li et al. 2008; Regales et al. 2009
	Lung	De Raedt et al. 2011
	Breast	Evers et al. 2010
	Prostate	Floc'h et al. 2012
	Prostate	Carver et al. 2011
	Pancreas	Olive et al. 2009
	Pancreas	Cook et al. 2012
	Pancreas	Jacobetz et al. 2012
	Pancreas	Provenzano et al. 2012
	Melanoma	Kwong et al. 2012
Coclinical	Lung	Chen et al. 2012
	Pancreas	Beatty et al. 2011
	Pancreas	Frese et al. 2012
Postclinical	Lung	Politi et al. 2010
	Breast	Rottenberg et al. 2007
	Breast	Rottenberg et al. 2008
	Breast	Jaspers et al. 2013

more accurate stromal composition compared to tumors engrafted in immunocompromised mice. Furthermore, the stepwise evolution of a nascent tumor from normal precursors will result in a more authentic tissue architecture compared to xenografts that are reconstituted from cell lines or are lacking in immune cells. Thus, GEMMs are well suited for studying the interplay between the tumor microenvironment and drugs.

One example of this comes from investigations into the underlying mechanism of chemoresistance in pancreatic cancer. Pancreatic ductal adenocarcinoma shows primary resistance to all classes of chemotherapy and targeted therapy. This phenotype is also apparent in a pancreatic cancer GEMM based on mutation of the endogenous *Kras* and *p53* genes in pancreatic cells, providingan excellent system for the study of primary chemoresistance. This model was used to show that stromal desmoplasia in pancreatic cancer results in poor vascularization and poor tissue perfusion (Olive et al. 2009). As a result, drugs are inefficiently delivered to pancreatic tumor tissues, resulting in reduced efficacy. This understanding, which has been supported by numerous contrast imaging studies in human pancreatic tumors, provides the rationale for stroma-targeted therapies as a means of facilitating the delivery of chemotherapeutic agents to pancreatic tumor tissues.

Even more compelling was a recent study of a CD40 agonist as a possible immune-targeted agent in pancreatic cancer (Beatty et al. 2011). Initial studies of the CD40 agonist were performed in pancreatic tumor xenografts and led to a proposed mechanism in which activation of CD40 resulted in activation of T-lymphoctes within the tumor. A clinical trial was performed based on these data and resulted in partial responses in 20% of patients. However, no evidence of T-cell infiltration of the treated tumors could be found, undermining the original hypothesis. A follow-up study in a pancreatic cancer GEMM found, instead, that the antitumor effect was mediated by macrophages, a hypothesis that was supported by subsequent analyses of human tumors. This compelling demonstration of the fidelity of a high-quality GEMM underscores the potential utility of the approach for understanding chemotherapeutic mechanisms.

With the development of GEMMs that accurately recapitulate aspects of human cancer, including the genetic alterations and natural history of the disease, it is likely that many agents will be tested in these models in preclinical, coclinical, and postclinical settings (see Table 2). The innate complexities of GEMMs make them ideal tools to study mechanisms of tumorigenesis and test the efficacy of old as well as new cancer therapies. As oncogenomic data from human tumors are paired with novel targeted agents, it is likely that GEMMs will play an increasingly prominent role in identifying the relevant patient population most likely to benefit from any given treatment.

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