

## The Use of Targeted Mouse Models for Preclinical Testing of Novel Cancer Therapeutics

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**Abstract** The use of genetically engineered cancer-prone mice as relevant surrogates for patients during the development of pertinent clinical applications is an unproven expectation that awaits direct demonstration. Despite the generally disappointing findings using tumor xenografts and certain early transgenic cancer models to predict therapeutic efficacy in patients, the dramatic progress of mouse models in recent years engenders optimism that the newest generation of mouse models will provide a higher standard of predictive utility in the process of drug development.

The purpose of drug development is to select, from millions of candidate compounds, those that most effectively and safely cure disease. Leading candidates proceed through a series of clinical phases designed to assess safety, dosing, and efficacy. This process is lengthy and expensive, with the cost for the entire clinical evaluation approaching hundreds of millions of dollars per drug (1). Additionally, only a minority of drugs that begin clinical assessment become approved therapies. Therefore, advances in our ability to optimally select candidate compounds for clinical evaluation are sorely needed.

To select lead compounds for clinical assessment, the pharmaceutical industry traditionally uses biochemical assays and cell-based proliferation and cytotoxicity screens. These assays are used to winnow compounds into reasonably sized subsets that have adequate pharmacokinetic properties and can be assayed in an *in vivo* animal efficacy model. Achievable concentration of drug dose, route of administration, and frequency of dosing are examples of critical variables that can only be derived from preclinical efficacy models, as they are basic characteristics of the compound chosen for clinical development.

Currently, the most commonly used animal models are tumor xenografts in immunodeficient mice. Xenografts are initiated through the injection of tumor cells from culture or through transplantation of a small tumor mass. Although the integrity of some molecular pathways may be conserved, cells propagated in two-dimensional cultures behave quite differ-

ently than an *in situ* tumor, and thus even carefully controlled orthotopic xenograft models may fail to fully recapitulate the behavior of the original malignant cells. One of the great advances in cancer biology over the past decade has been the recognition of the dynamic interactions that take place between tumor and host (2). Tumor cells are the subject of both negative and positive signals from a variety of sources, including stromal cells, matrix proteins, endothelia, immune cells, and perhaps neighboring epithelial cells. When a tumor is removed from its native site, these complex interactions are interrupted. Those cells that survive and proliferate, whether transplanted *in vivo* or propagated in a tissue culture dish, may be quite distinct from their initial state and not representative of the original heterogeneity present in the tumor. This is not to say that tumor explants are of no value, but the traditional xenograft approach compromises the ability to assess the complete role of non-cell autonomous components during therapeutic investigations.

This highlights the most important concept in preclinical modeling: predictive utility. How effective is a particular model at selecting efficacious drugs? How frequently do drugs that succeed in a preclinical assay subsequently fail when administered to human patients? Unfortunately, neither cell-based assays nor xenograft models are particularly successful in predicting drug responses in humans. A broad analysis of *in vitro* models and tumor xenografts done at the National Cancer Institute found poor correlations with activity in phase II clinical trials and generally concluded that only compounds that are successful in a large number of different models are likely to be effective in the clinic (3).

Genetically engineered mouse models (GEM) are a promising alternative to traditional preclinical assays. When appropriately designed, they may address many of the shortcomings of cell-based assays and xenografts. GEMs provide *in situ* tumor development in an immunocompetent animal setting. However, not all GEMs are appropriate for the purpose of preclinical drug testing, and general acceptance of these models as preclinical tools has been hesitant due to the mixed results previously obtained.

Transgenic mice that ectopically express viral or cellular oncogenes were the first type of GEM produced, and although informative for certain investigations [such as the predicted efficacy of vascular endothelial growth factor receptor blockade

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in a mouse model of insulinoma based on the SV40 large T antigen (RIP-Tag); ref. 4), they have provided conflicting results in many other contexts. For example, farnesyltransferase inhibitors were developed as inhibitors of Ras processing (5), and although farnesyltransferase inhibitors showed exceptional potency in causing regression of mammary gland tumors in transgenic mice ectopically expressing the *HRAS*<sup>G12V</sup> oncogene in the mammary epithelium (6), these results did not predict the overall clinical failure of farnesyltransferase inhibitors in patients suffering from neoplasms that harbored *RAS* mutations. Interestingly, upon further investigation, farnesyltransferase inhibitors did not show preclinical efficacy in GEMs that activated the Ras pathway due to deficiencies in NF1 (7). This suggests that GEMs, based on a physiologic genetic context, may be more suitable for certain preclinical therapeutic investigations.

The purpose of this article is to discuss the use of targeted mouse models in a preclinical setting and to propose criteria for the evaluation of their success. This work will also consider issues that are unique to working with spontaneous mouse models, such as tumor detection and imaging, drug trial structures, and the use of mouse models as agents for drug discovery. These issues will be considered in the context of a recently developed preclinical model of pancreatic cancer that is currently being evaluated in our laboratory.

## Pancreatic Ductal Adenocarcinoma

The need for novel therapeutics directed against pancreatic ductal adenocarcinoma (PDA) is great. The 1-year survival rate for untreated PDA is only 19%, resulting in an annual incidence of new PDA cases that closely matches the annual death rate of ~32,000 people per year in the United States (8). Even among patients who are appropriate surgical candidates, most eventually succumb to locally recurrent and metastatic disease (9). For patients with nonresectable PDA, the current standard therapy is gemcitabine (Gemzar, Eli Lilly Co., Indianapolis, IN), a genotoxic drug that extends life by a matter of weeks. Although some patients do respond favorably to gemcitabine, most do not and no clear basis for the stratification of these two groups has been determined.

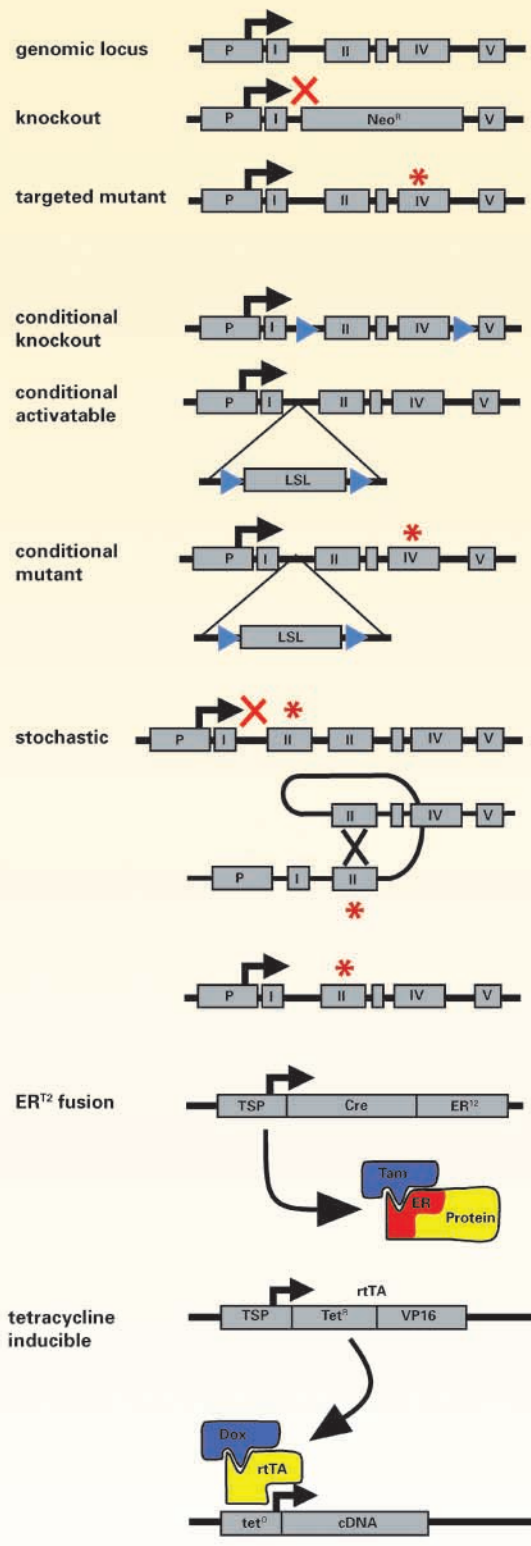
Studies of human PDA samples have delineated a number of common genetic alterations in PDA. Principle among them are activating mutations in the *Kras* proto-oncogene, which are found in nearly 95% of human PDA (10, 11). *Kras* is a small GTPase that receives signals from receptor tyrosine kinases. Mutations at codons 12, 13, 59, 61, or 63 in *Kras* impair its intrinsic GTPase activity and confer insensitivity to cytosolic GTPase-activating proteins, thereby "locking" the enzyme into an active *Kras*-GTP conformation for signaling through a variety of effector pathways involved in cell proliferation, growth, and survival (12). Several tumor-suppressor pathways have been implicated in PDA progression (13). The *Ink4a* gene locus, which encodes the cyclin-dependent kinase inhibitor p16<sup>Ink4a</sup> and the tumor suppressor p14<sup>ARF</sup>, is very commonly mutated or methylated in PDA. Likewise, *DPC4*/*SMAD4*/*MADH*, a mediator of the transforming growth factor- $\beta$  pathway, is also inactivated at high frequency. Also, the *p53* tumor suppressor gene, a transcription factor that activates arrest and apoptosis pathways in response to diverse genotoxic and oncogenic stimuli, is mutated in ~70% of advanced

tumors (14). Finally, in addition to these genetic alterations, up-regulation of the receptor tyrosine kinase epidermal growth factor receptor (15), the Rho-family GEF VAV1 (16), and activation of the Notch (17) and sonic hedgehog pathways (18, 19) have recently been shown to be common events in pancreatic tumorigenesis.

These molecular alterations manifest in the distinctive histologic changes that define cancer: loss of differentiated features such as cell polarity, increased nuclear to cytoplasmic ratio, nuclear pleiomorphism, aberrations in cell division, loss of tissue organization, invasion, and metastasis. As with many epithelial cancers, these changes occur in an ordered, stepwise progression that correlates with the acquisition of particular genetic mutations. Two distinct precursor lesions have been recognized for ductal pancreatic cancer: pancreatic intraepithelial neoplasia (PanIN) and intraepithelial papillary mucinous neoplasms (IPMN; ref. 20). PanINs are microscopic proliferations of the smaller pancreatic ducts and proceed in a spectrum ranging from the fairly common and benign PanIN-1a lesion through PanIN-3, a carcinoma *in situ*. A more varied collection of macroscopic lesions make up IPMNs, which form in the larger pancreatic ducts. In addition to the histologic distinctions between PanINs, and IPMNs, emerging evidence suggests that they harbor distinct molecular determinants as well. For example, alterations in *p53* and *DPC4* are more common in PanINs than in IPMNs whereas the reverse is true for the expression of *MUC2*, a type of mucin normally expressed in the intestine. In both PanINs and IPMNs, activating mutations in *Kras* are among the earliest and most prevalent changes. Therefore, many of the strategies for modeling PDA have focused on this gene.

## Constructing Preclinical Cancer Models

Human tumors are thought to develop through the accumulation of multiple mutations that predispose to increased survival, growth, and dissemination. These genetic alterations occur spontaneously within somatic cell genomes and in those cases where mutant proteins are produced, they are expressed at physiologic levels from endogenous promoters. Although some genes are dramatically up-regulated in tumor cells, this process still occurs within the constraints of mammalian genetics. Following these principles, there is now an extensive toolkit with which to craft an accurate model of cancer (Fig. 1). For the purposes of preclinical modeling, the most compelling are those that manipulate the endogenous genome to effect mutations that closely mimic the state of human tumors. These include knockout alleles, in which a gene is deleted, as well as targeted mutant alleles, which harbor subtle mutations in the endogenous locus. Both knockout and targeted mutant alleles are useful for modeling hereditary tumor syndromes that result from the loss of one copy of a tumor-suppressor gene (e.g., hereditary retinoblastoma; refs. 21–23), Cowden's disease (24), or the subtle mutation of an oncogene or tumor-suppressor gene (e.g., familial *GIST*; ref. 25), Li-Fraumeni syndrome (26, 27). However, many such alleles result in embryonic lethality or background tumor spectra and thus are not ideally suited to modeling spontaneous cancers. Therefore, conditional alleles have been developed that allow controlled deletion, reactivation, or mutation of



**Fig. 1.** Genetic intervention strategies for preclinical mouse modeling. Strategies for manipulating endogenous gene loci in mice are depicted for a hypothetical gene. P, endogenous promoter; arrows, recombinate recognition site, such as Lox P; \*, point mutation; LSL, Lox-STOP-Lox cassette (a gene silencing element); TSP, exogenous tissue-specific promoter; Cre, bacterial recombinase cDNA; ER<sup>T2</sup>, estrogen receptor fusion.

endogenous genes. This can be achieved through the incorporation of bacterial recombinase systems, such as Cre/lox (28), Fip/frt (29), or Dre/rox (30). These enzymes catalyze either the excision or inversion of sequences flanked by associated recognition sites, depending on the relative orientation of the sites. By driving recombinase expression from a tissue-specific promoter, one can restrict gene deletion or expression to desired tissues. Alternatively, the recombinase may be delivered by viral vector or protein transduction.

Conditional recombination alleles may be abstracted a level further by the incorporation of drug-sensitive elements. Regulatory elements sensitive to tetracycline or tamoxifen analogues may be used to achieve a level of temporal control (31, 32). This is particularly useful in systems where the tissue-specific genes used for spatial restriction are expressed early in development, potentially hitting primitive cells unrelated to the origin of the targeted tumor type. Drug-inducible systems have the added advantage of being dose sensitive, potentially allowing for control of tumor number and latency.

One drawback to systems that rely on a tissue-specific promoter is that the resulting mutated cells are surrounded by other mutant cells. In a spontaneous human tumor, the initiating mutation likely occurs in a cell that is surrounded by normal cells. This effect is mimicked by latent alleles that rely on the stochastic homologous recombination of a gene segment duplication to activate an endogenous mutant gene (33). This strategy could be useful for investigations into tumor surveillance but is currently hindered by a lack of simple techniques for assessing which cells have undergone rearrangement.

## Mouse Models of PDA

A number of mouse models of pancreatic cancer have been developed in the past few years, providing a wealth of information about the developmental and genetic etiology of PDA (34). Our group developed two models of PDA that bear striking resemblance to the human condition. The first is based on mutation of the endogenous murine *Kras* gene specifically in pancreatic progenitor cells (35, 36). This was achieved by crossing mice with a conditional activated *Kras* allele (LSL-*Kras*<sup>G12D</sup>) to either of two transgenic strains that express Cre recombinase in pancreatic lineages (PdxCre or p48Cre). These "KC" mice develop murine PanIN with 100% penetrance (35). Furthermore, a subset of these mice developed PDA tumors at an advanced age, suggesting that additional events were necessary before tumor formation could proceed. To accelerate this process, PdxCre-expressing compound mutant animals were generated with conditional mutations in both *Kras* and *Trp53* (27, 37). These "KPC" animals developed advanced PDA with 100% penetrance at an early age. Furthermore, KPC mice recapitulated many aspects of the human disease, including histopathologic similarities in neoplastic tissue, the common occurrence of metastasis to relevant sites, comorbidities such as cachexia, activation of biochemical pathways, and evidence for genomic instability.

## Evaluating Preclinical Models of Cancer

There are three distinct uses for mouse models of cancer: as an aid in the investigation of the basic biological principles of

cancer, as an assay for the preclinical development of anticancer drugs, and as a tool for discovering new clinical agents and assays. Over the past 10 years, mouse models have primarily been used for basic research, yielding great advances in the understanding of tumor biology. In this time, GEMs have improved in their sophistication and faithfulness to the genetic lesions observed in human tumors. Concurrently, their success in recapitulating the phenotypes of targeted tumors has also improved. However, to avoid the experiences of previous preclinical assays, additional layers of rigor and fidelity should be demanded of mouse models that are to be used in a preclinical setting. The role of the preclinical mouse model is to stand in place of human patients. Therefore, it is essential to always be guided by the human disease.

The following features should be considered in assessing the utility of a mouse model for preclinical studies. First, genetic manipulations should accurately reflect the genetics of the human disease. This includes both the genetics of the targeted cells as well as nontargeted cells. The ideal model will produce subtle, controlled mutations in relevant endogenous genes in targeted cells, while leaving an effectively wild-type genotype in nontargeted cells. Any weaknesses in the genetic strategy should be acknowledged so that one can be alert for subsequent phenotypic deviations. For example, the use of conditional mutant alleles in the KPC model results in constitutional heterozygosity for *Kras* and *Trp53* in the non-Cre-targeted cells. This is particularly relevant given recent reports of mutations in *Trp53* and other tumor-associated genes in the stromal cells of epithelial tumors (38). Although not necessarily diminishing the usefulness of this model, we nonetheless should be cognizant of the potential for non-cell-autonomous effects of haploinsufficiency.

Second, the histology of the model should closely reflect that of human tumors. Nearly all of the early models of cancer involving viral oncoprotein overexpression produced histologies distinct from that of common human tumors. Carefully validating the histology of a model should be done by clinical pathologists with particular expertise in human pathology and veterinary medicine. In addition, associated pathophysiologic conditions, such as cancer cachexia, should also be assessed.

The third step of model validation is to explore the tumor phenotype at a molecular level. This should include an assessment of gene expression, with particular emphasis placed on known tumor markers from human studies, as well as an analysis of genetic and genomic alterations frequently seen during tumor progression in humans.

Finally, because the predictive utility of a model can only be established in retrospect, we believe it is important to establish a new criterion for the analysis of preclinical models, which we will refer to as "credentialing." Credentialing a model involves administering to the mice those drugs that have previously been tested in human patients. The utility of this experiment is clear when modeling tumors for which an effective therapy already exists: If a model responds differently than do human patients, then its predictive utility for that agent is poor. However, we believe this experiment should also be done even when no effective therapies are currently available to measure the selectivity of the model against ineffective therapeutics. For example, standard pancreatic xenograft models respond quite well to treatment with gemcitabine (39), despite its rather

limited efficacy in human patients. Such a positive response to ineffective therapeutics should severely question the utility of that model for preclinical investigations.

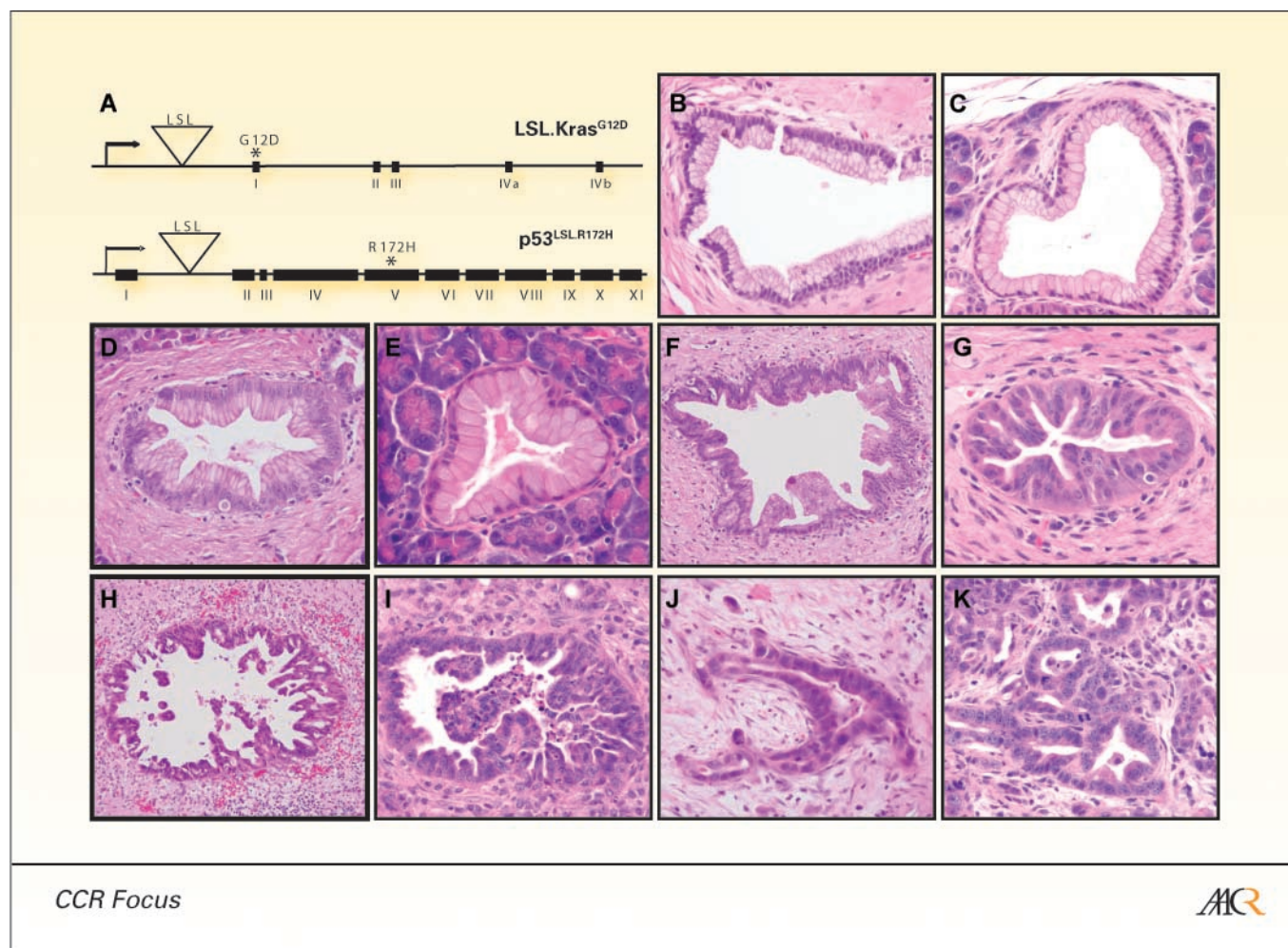
The KC and KPC models of pancreatic cancer have been validated in a number of ways. Both develop the full range of PanIN lesions seen in humans and both progress to overt carcinoma (Fig. 2). The KPC model in particular develops tumors with ductal morphology, abundant stroma with collagen deposition and associated comorbidity such as jaundice, cachexia and ascites. Preinvasive and invasive lesions from both models express specific mucins and the ductal marker cytokeratin 19. In particular, MUC5a/c, a mucin that is absent from normal ductal cells, is abundantly expressed in PanINs and PDA from these mice. Several proteins found up-regulated in human tumors have also been observed in these models, including the Notch pathway effector Hes1, cyclooxygenase 2, matrix metalloproteinase 7, sonic hedgehog ligand-1, Her2/neu, and epidermal growth factor receptor. Some of these markers are found early in tumor progression,

whereas others accumulate in a stochastic manner later in tumorigenesis. Finally, evidence of genomic instability, a common finding in human PDA, was apparent in KPC tumors and derived cell lines.

### Tumor Imaging in Mouse Models of Cancer

With spontaneous mouse models, it is often unclear which animals have developed overt tumors. For example, KPC mice survive between 2 and 10 months before succumbing to PDA. Simply choosing a time point at which to enroll the animals would result in treating numerous mice that do not harbor tumors and necessitate the use of much larger animal cohorts. The alternative is to identify an effective noninvasive method to detect early PDA tumors.

All of the imaging modalities commonly used in cancer patients have been adapted for use with small animals, including magnetic resonance imaging, computed tomography, positron emission tomography, single photon emission



**Fig. 2.** The KPC model of PDA. *A*, conditional mutant *Kras* and *p53* alleles used in the KPC model to restrict expression of the endogenous mutant proteins to the pancreas. A loxP flanked gene silencing cassette (*LSL*) was inserted into upstream promoter or intronic sequences. This cassette is excised by the action of Cre recombinase, expressed under the control of either the *Pdx1* or *p48* promoter. *B* to *K*, side-by-side examples of human (*B*, *D*, *F*, *H*, and *J*) and KPC mouse (*C*, *E*, *G*, *I*, and *K*) pancreatic cancer pathology. These include PanIN-1A (*B* and *C*), PanIN-1B (*D* and *E*), PanIN-2 (*F* and *G*), PanIN-3 (*H* and *I*), and PDA (*J* and *K*).

**Table 1.** Comparison of common tumor imaging modalities for use with small animals

	MRI	CT	PET	SPECT	BLI	BFI	US
Equipment size	Large	Small	Small	Small	Small	Small	Small
Resolution	High	High	Low	Low	Low	Low	High
Session time	Hours	Minutes	Hours	Hours	Minutes	Minutes	Minutes
Provides functional information	Not usually*	No	Yes	Yes	Yes	Yes	Not usually <sup>†</sup>
Staffing/training requirements	High	Intermediate	Intermediate	Intermediate	Low	Low	Intermediate
Requires engineered strains of mice	No	No	No	No	Yes	Yes	No
Approximate capital cost	>\$1 million	\$100K-250K	~\$250K	~\$250K	\$100K-250K	\$100K-250K	\$200K

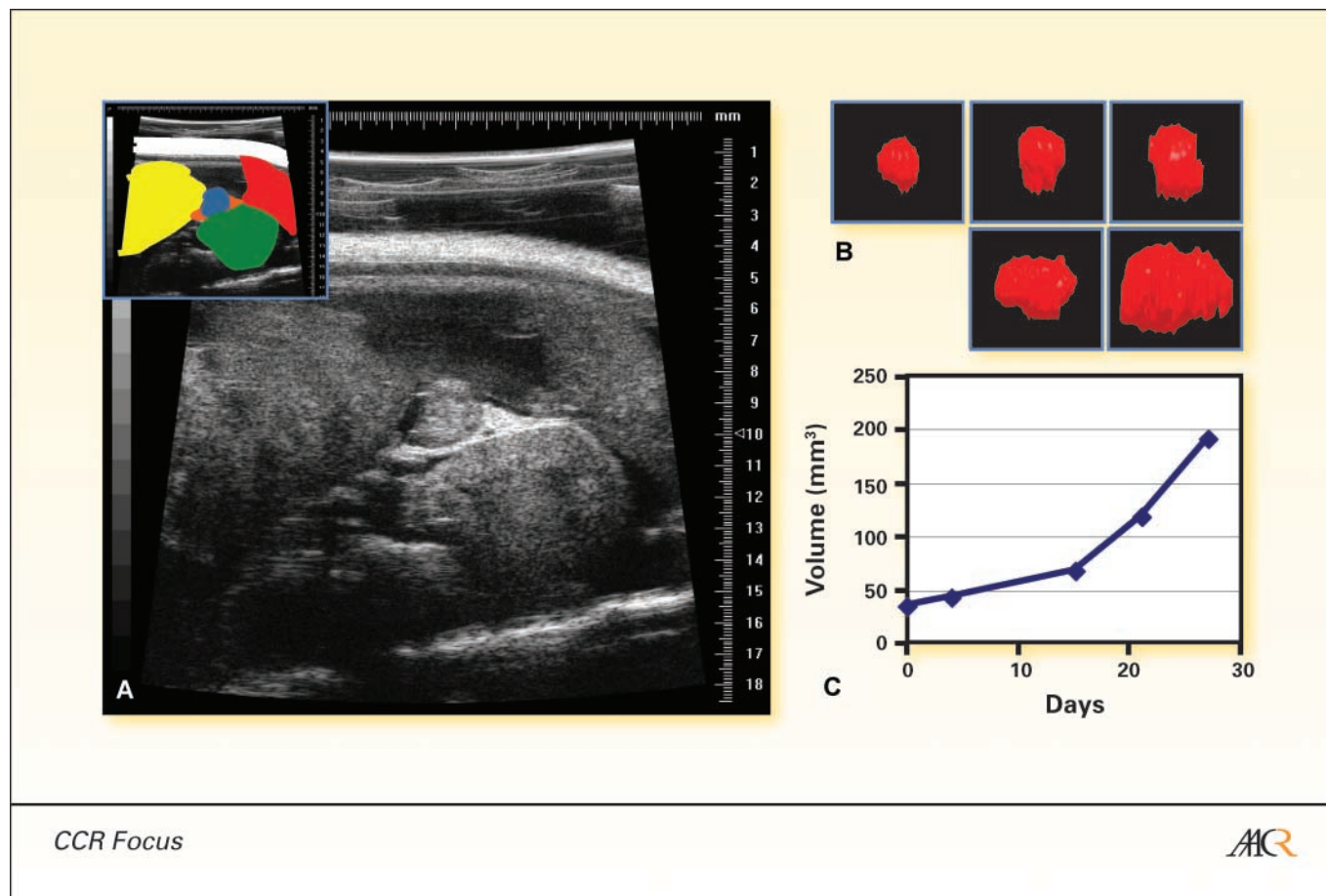
Abbreviations: MRI, magnetic resonance imaging; CT, computed tomography; PET, positron emission tomography; SPECT, single photon emission computed tomography; BLI, bioluminescent imaging; BFI, blood flow imaging; US, ultrasound.

\*Advanced applications may incorporate magnetic particles or other contrast agents to provide functional information.

<sup>†</sup> Doppler ultrasound provides information on blood flow.

computed tomography, and ultrasound. Additional modalities based on genetically engineered alleles are also available, including optical imaging technologies that detect fluorescence or luminescence. Each of these approaches has its own advantages and limitations (Table 1), and several features should be taken into account when exploring imaging options.

An issue of great practical relevance is whether the equipment for that technology can be made available inside an animal barrier facility. Preclinical studies require repeated imaging of the same animal over time and this will be encumbered if the animals must be removed from the facility to be imaged. Another consideration is the session time: Longer than 30



**Fig. 3.** Noninvasive imaging of *in situ* PDAs by high-resolution ultrasound. *A*, raw image from a KPC mouse with a 9-mm-diameter PDA. Color-coded inset key, tumor (yellow), spleen (red), left kidney (green), adrenal gland (orange), and an invading tumor nodule (blue). *B*, three-dimensional reconstructions of a representative tumor at five different time points. *C*, calculated volumes from an imaged tumor plotted over time.

minutes per mouse becomes impractical when running larger studies. In general, the optimal imaging procedure should be brief and minimally invasive.

A principal use of therapeutic imaging is to observe and quantify tumor mass or "tumor activity" during preclinical investigations. Anatomically, this may be achieved by three-dimensional reconstruction of two-dimensional slices (computed tomography) or by quantification of a signal produced by the tumor in direct proportion to its size. For example, luciferase reporter systems are well suited to quantifying tumor size provided expression is limited to tumor cells. Optical imaging systems, as well as some nuclear techniques, such as positron emission tomography and single photon emission computed tomography, have the additional capacity to provide functional information about the biology of the tumor. For example, several strains have been constructed that use luciferase reporters with transcription factor response elements. This strategy was used to monitor activation of E2F1 (40) and also as a sensor of physiologic processes, such as hypoxia and angiogenesis (41).

The most effective methods for clinical diagnosis of pancreatic cancer are endoscopic ultrasound and computed tomography. The physics of ultrasound incur a trade-off between greater depth of penetration (at lower frequencies) and higher resolution (at higher frequencies). In endoscopic ultrasound, a small ultrasound transducer is passed down the esophagus to provide high-resolution local imaging of the pancreas, generally at a range of 4 to 8 MHz. The sensitivity of endoscopic ultrasound for detecting lesions has been unmatched by other modalities. However, computed tomography technology has advanced rapidly in recent years and may now provide higher specificity in the diagnosis of resectable versus unresectable disease. These techniques together provide very high rates of both sensitivity and selectivity (42).

To image the KC and KPC models, we have chosen to pursue high-resolution ultrasound as a noninvasive means of imaging. Due to the small size of a mouse, extremely high resolution may be achieved from a 35 MHz transducer (VisualSonics, Inc.), while still maintaining a deep enough field of view to image the entire abdominal cavity. This system offers the advantage of short session time (~15 min/mouse), high resolution with the ability to reconstruct and quantitate tumor volumes, and small instrument size to enable the placement of the ultrasound unit directly in our animal room. Using ultrasound, we have been able to image and quantify tumors over time following the detection of lesions as small as 1 mm in diameter (data not shown). Figure 3 provides an example of an ultrasound image of a tumor from a KPC mouse.

## Trial Design

**Pharmacokinetics and pharmacodynamics.** In patients with solid tumors, the pharmacokinetic properties of a drug are infrequently determined in the actual target tissues. Serum or plasma samples are available to determine certain pharmacokinetic properties, but it is usually not possible to obtain tumor material during treatment. Mouse models make available this option and can provide crucial information on whether an

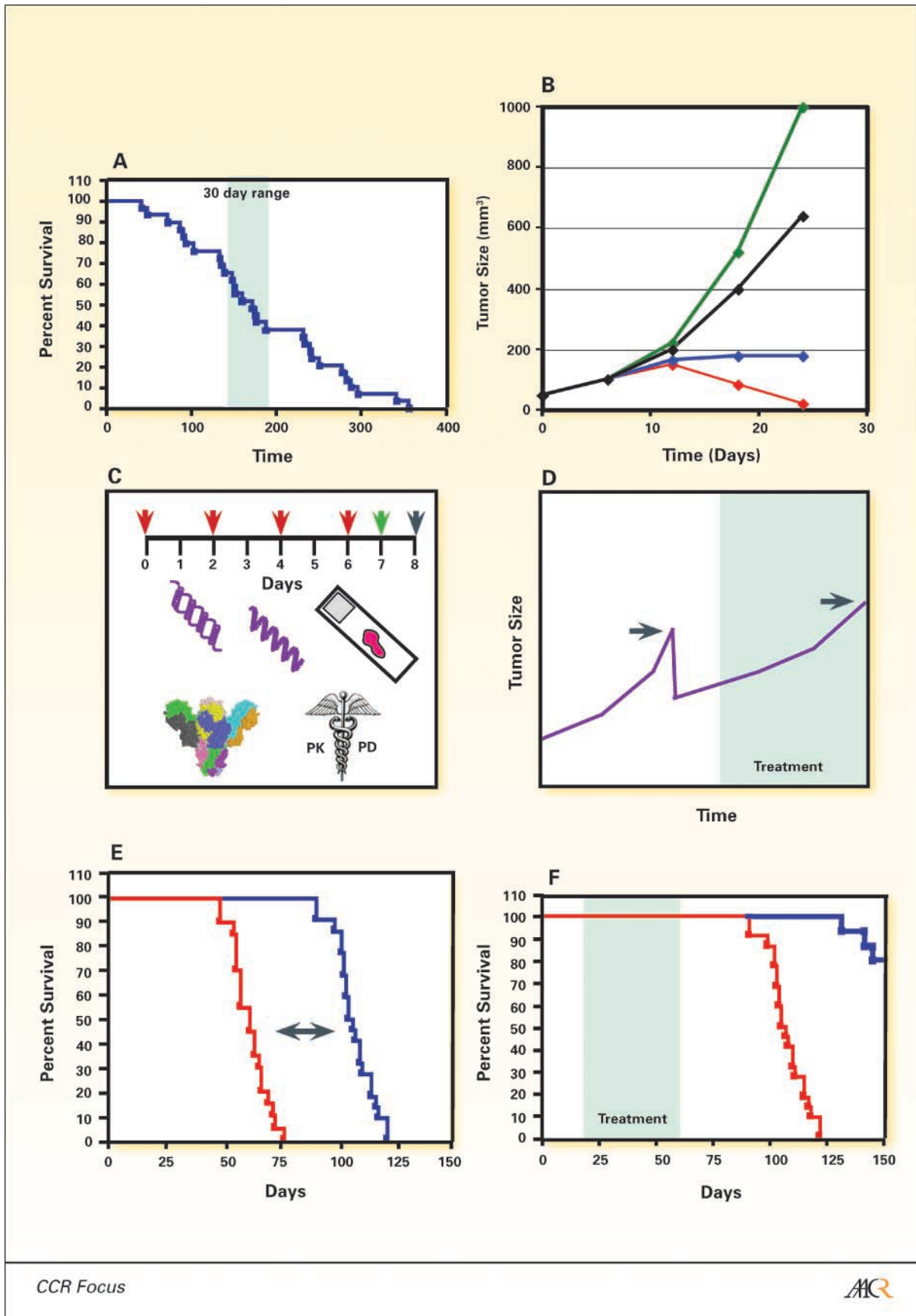
agent is biologically available in the target tissue. Although drugs may be metabolized differently in mice and humans, these data will provide a baseline for the assessment of drug efficacy in the preclinical model. To this end, pilot studies should be designed to assess both the turnover of drug in the blood and the bioavailability of the drug in the target tissue and tumors.

It is also desirable to determine the pharmacodynamic effect of a drug on its target tissues. For targeted therapeutics in particular, early pilot studies should be done to determine whether the drug is successful in perturbing the targeted pathway in tumor cells. This is routinely achieved through immunohistochemical and expression profiling techniques, although other approaches are possible. As mentioned for pharmacokinetic studies, there may be important differences in pharmacodynamic characteristics when comparing mouse models to patients, so each case will need to be rigorously studied. The collection of pharmacodynamic and pharmacokinetic information, as well as the determination of maximum tolerated dose for mice, will aid in preclinical trial design, particularly in dosing and delivery.

**Time point versus image-based enrollment.** There are several variables to consider when designing a preclinical trial that will be influenced by the goal of the study and the nature of the model (Fig. 4). The first is whether enrollment is based on time point or radiographic detection. The latter option is preferable for any model that has a variable latency or penetrance, a common concern with many genetically engineered mice. Imaging modalities allow animals to be enrolled with tumors of a particular size or location, helping to reduce the variability between subjects. This approach allows the growth of the tumor to be tracked before and during treatment, individualizing the preclinical therapeutic experiment in a similar manner to the clinical setting. For models with a precipitous and well-defined survival curve, it may be feasible to enroll at a particular age. For this approach, it is advisable to carefully assess tumor development in a large cohort of untreated animals both at end point and at various time points so as to provide a thorough picture of the kinetics and variability of tumor development.

**Short-term versus long-term intervention.** A wide array of information can be acquired from intervention studies using preclinical models, ranging from efficacy assessments of survival prolongation to the ability of an agent to perturb particular molecular pathways. Effect on survival time may be assessed through a long-term intervention study, wherein enrolled subjects are treated either for a defined period of time or indefinitely. In this setting, imaging can be used to track tumor progression, stabilization, or remission. This approach is the most similar to clinical oncology: Each mouse acts as an individual cancer patient and yields all of the same information available to the oncologist.

Short-term interventions are useful for assessing the molecular effects of a drug and for determining pharmacokinetic and pharmacodynamic variables. Animals are enrolled and treated for a short period of time and then euthanized to provide materials such as DNA, RNA, protein, chromatin, plasma, serum, or tissue sections. If a model produces a great deal of heterogeneity in tumor behavior and molecular pathogenesis, it may even be desirable to submit the animals to survival surgery





to acquire a biopsy of tumor tissue before treatment. Following surgery, the animal is treated for a short period of time and then euthanized, providing a powerful matched set of samples from the same tumor before and following treatment.

Finally, tumor prevention studies may be carried out to assess the ability of a therapeutic to prevent the development of cancer in the first place. It must be noted that some mouse models are ill-suited to this purpose. In cases where an entire tissue is subject to mutation, resulting in multifocal, heterogeneous tumor development, it may be difficult to assess the success of a chemopreventative agent.

### Practical Considerations in the Use of Mouse Models for Preclinical Testing

A number of practical issues should be considered when carrying out preclinical studies with mouse models. In most cases, breeding the animals will be the rate-limiting step, so breeding ratios in the final generation should be kept as low as possible. This can often be facilitated by harboring alleles in homozygous fashion in the parents so that complex crosses may be made more manageable. For example, generating KPC mice requires a 1:8 cross if heterozygous parents are used; yet, this can be improved to a 1:2 cross through a single extra generation of breeding LSL.K-ras<sup>G12D/+</sup>; LSL-Trp53<sup>R172H/R172H</sup> males crossed to homozygous PdxCre females. Genotyping can also become rate- and cost-limiting, thus it is worth considering outsourcing this to services that specialize in mouse genotyping. Alternatively, for single allele models, it may be desirable to include a coat color marker into the targeted locus, obviating the need for molecular genotyping (43).

When running a trial, rapid techniques for health monitoring are useful, particularly with traditional chemotherapies or other highly toxic regimens. Complete blood counts are informative but the equipment can be prohibitively expensive. Thus far, the most common monitoring techniques are hematocrits, daily weight measurements, and direct behavioral observation.

Finally, methods of drug delivery should be taken into account. S.c. injection, i.p. injection, and oral gavage are all appropriate for routine use although the effect of delivery route on drug metabolism should be considered (e.g., rapid clearance and metabolism of drugs by the liver following i.p. injection). Additionally, i.v. delivery using intermittent tail vein injections or long-term venous catheterization is possible with appropriate training. Finally, micro-osmotic pumps that can deliver a constant dose of drug over a period of hours or days are commercially available for implantation into mice. This is particularly useful for drugs with a short half-life.

### Drug Discovery and Development with Mouse Models

Preclinical mouse models may be exploited to accelerate drug discovery. For example, they offer the opportunity for rapid validation of potential drug targets. This can be assessed on a candidate basis by engineering strains with targeted mutations in genes that may be potential drug targets and combining them with established tumor models. Matrix metalloproteinases were identified as potential drug targets through their targeted disruption in RIP-TAg mice (44). Such “genetic intervention” models illustrate the potential outcome of a completely potent drug and will influence the decision of whether to pursue these targets pharmacologically. Alternatively, short hairpin RNA knockdown strains can be rapidly engineered via lentiviral infection of embryonic stem cells and have the potential to produce an allelic series of strains with a gradient of protein levels for the targeted gene (45). This may mimic the effect of partial inhibition of a target protein.

Mouse models are also well suited for the discovery of biomarkers for the detection of early tumor lesions or for the rapid detection of response to treatment. Biomarker discovery from clinical specimens is challenging due to the pronounced heterogeneity of genetic and environmental background among human populations. In contrast, mice exist in a controlled environment and may be inbred to homogeneity. Furthermore, large numbers of early preneoplastic samples can be collected from otherwise healthy mice, a task that is extremely challenging for many types of human tumors. For these reasons, genomic and proteomic analyses of mouse models provide a great opportunity to detect relevant biomarkers. Indeed, serum marker analysis of KC mice has already yielded a distinct proteomic signature for animals with PanIN lesions compared with healthy animals or those harboring PDA (35). That such findings could be translated to human samples was shown through work on a related mouse model, which used expression profiling of murine lung adenocarcinomas to uncover a previously cryptic signature of *Kras* mutation in a human data set (46). Approximately 80% of patients diagnosed with PDA present with advanced disease, so the identification of a predictive biomarker for early pancreatic cancer would represent a truly monumental advance in the field.

Primary cells and cell lines established from targeted mouse models of cancer provide a unique, genetically defined reagent for drug development and discovery. Besides investigating lead compounds known to be specific for given molecular targets, this approach can be extended to chemical library screening to isolate novel candidate compounds. Another variation is to interrogate genetically defined cell lines with lentiviral libraries as a means of identifying drug targets (47). Likewise, information may be

**Fig. 4.** Trial designs for preclinical experiments. *A*, actual survival data from KPC mice. Green highlight, a hypothetical 30-day period of time, illustrating the small subset of animals that might be expected to harbor tumors during a time point – based enrollment study. *B*, hypothetical tumor volumes over time. Using an image-based enrollment strategy, the growth of each tumor can be plotted, allowing for the detection of disease acceleration (*green*), stabilization (*blue*), or regression (*red*), compared with control tumor growth rate (*black*). *C*, structure of a hypothetical short-term intervention trial. Animals are treated with drug every other day for 8 days (QOD×4; *red arrows*). Twenty-four hours following the final dose, animals are treated with bromodeoxyuridine to aid in proliferation analyses and then euthanized the following day. At necropsy, tumor and normal tissues are collected to provide DNA, RNA, protein, histology, and other materials for analysis of drug pharmacokinetics and pharmacodynamics. *D*, structure of a hypothetical study based on survival surgery. Survival surgery may be used to acquire pretreatment and posttreatment tissue (*arrows*) from the same tumor, allowing for a careful analysis of drug effects on tumor biology. *E*, structure of a hypothetical long-term intervention study of image-enrolled mice. Graph depicts survival following image-based tumor detection. Treatment commences when the tumors reach a predetermined size and may continue for a set period of time or indefinitely. Use of image-based enrollment generates a steeper survival plot and can dramatically reduce the number of animals necessary to detect a change in survival time. *F*, structure of a hypothetical prevention study. In a prevention trial, animals are treated before the detection of disease and assessed for a change in survival.

gleaned from analysis of mutational events that occur at different stages of tumor progression. Powerful sets of samples can be generated from laser capture microdissection of tumor sections at defined stages and these can be analyzed through a wide variety of different genetic and genomic platforms.

## Conclusions

Targeted mouse models harbor great promise for accelerating the drug discovery process. By incorporating a predictive tool into the preclinical screening process rather than awaiting the results of phase II trials, it will be possible to screen more potential therapeutics in a meaningful manner. Furthermore, mouse models are an ideal platform for testing combinations of therapeutics. Combination testing in humans is encumbered for both practical and proprietary reasons. Yet, there is every reason to believe that certain combinations of drugs will be effective even when each of the individual agents have no effect (thereby precluding Food and Drug Administration approval in the first place). Mouse models offer the opportunity to test the combination therapy hypothesis and perhaps provide the data necessary to change how clinical drug testing proceeds.

The current preclinical pipeline follows a fairly predictable course. Cellular experiments are done to determine the ability

of a compound to effect a functional change, whereas biochemical studies are carried out to determine its pharmacodynamic effect. Following this, medicinal chemistry efforts are pursued to identify compounds with optimal pharmacokinetic characteristics, while preserving or improving the pharmacodynamic properties. Finally, toxicity studies are done in two additional mammalian species and efficacy studies are carried out in xenografts. If targeted mouse models prove more successful at predicting clinical response than xenografts, then it is logical that they will replace xenografts in this process. Additionally, we suggest that the most predictive models be integrated early in the drug development process rather than late—before chemical optimization and toxicity assessment. Each animal test provides a wealth of related data that should inform and direct future experiments, and less time and effort should be wasted on ineffective compounds and thereby accelerate the overall rate of drug discovery for cancer patients.

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