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Primer
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Preclinical Mouse Cancer Models: A Maze of Opportunities and Challenges

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Significant advances have been made in developing novel therapeutics for cancer treatment, and targeted therapies have revolutionized the treatment of some cancers. Despite the promise, only about five percent of new cancer drugs are approved, and most fail due to lack of efficacy. The indication is that current preclinical methods are limited in predicting successful outcomes. Such failure exacts enormous cost, both financial and in the quality of human life. This Primer explores the current status, promise, and challenges of preclinical evaluation in advanced mouse cancer models and briefly addresses emerging models for early-stage preclinical development.

Explosion of Cancer Therapies and Challenges to Clinical Success

Ever-increasing knowledge of cancer biology has yielded countless possibilities for diagnostic and therapeutic strategies (Figure 1), while at the same time revealing enormous disease complexities that challenge clinical success. Such challenges include tumor microenvironment complexities, intra- and inter-tumor molecular and biological heterogeneity, systemic and tumoral immune and metabolic response heterogeneity, and the ability of drug-resistant stem-like cancer-initiating cells to repopulate treated cancers (Pattabiraman and Weinberg, 2014). Too often, experimental targeted therapies designed to assimilate known disease complexity have proven ineffective, only to highlight the limitations in our understanding. In contrast to most experimental targeted therapies, encouraging advancements have been made using a number of cell-based and targeted immunotherapies, which have produced sustained responses in patients (Page et al., 2014). However, only a fraction of patients respond to these therapies.

Over the last decade, cancer classification has shifted from relying solely on histiopathologic properties to including key molecular attributes that can predict therapeutic outcomes. That certain molecular aberrations are targets for effective therapy first led to clinical practice in 1995 after a leukemia (APL) bearing the PML-RARα translocation was shown to be sensitive to retinoic acid (tretinoin) (Quignon et al., 1997), which targets the RARα component to effect leukemic cell differentiation. Soon thereafter, Herceptin (a Her2 inhibitor) was approved for treating Her2+ breast cancer (1998), and Gleevec (a BCR-ABL inhibitor) was approved for CML treatment (2001). These highly effective drugs rapidly became the standard of care. Although these successes establish the promise of targeted therapies, most attempts to attain similar results targeting known molecular drivers have failed, and the reasons are often elusive because of human research limitations. Some general principles have been recognized that emphasize the need for preclinical platforms approximating human cancers. For example, in each of the noted successes, single potent cancer drivers present in a significant fraction of patient malignancies were targeted; however, when a minor fraction of patients are responsive, all-comer clinical trial data may mask the responders. This was first demonstrated in non-small-cell lung cancer (NSCLC) patient trials that initially failed to show significant responsiveness to EGFR-targeted tyrosine kinase inhibitors; however, the ~10% of patients whose tumors actually harbored activating EGFR mutations were uniquely sensitive (Lynch et al., 2004; Paez et al., 2004). Now, screening of lung cancers for such mutations prior to therapy is routine practice. Lung cancer is the most prevalent US cancer; if limited to clinical trials, accurate identification of therapies effective in a fraction of less-common cancer types may not be possible. Nonetheless, when a specific target was known, stratification of patients has identified additional effective therapies, such as inhibitors for BRAF mutant melanomas and ALK translocation-positive NSCLCs (Pagliarini et al., 2015). Unfortunately, patients treated with single targeted therapies inevitably relapse with cancers that are resistant to the original drug.

Another challenge in targeting single drivers is the feedback response upon molecular network disruption that prevents efficacy or causes increased severity. Understanding such molecular responses can aid in the discovery of more effective combination therapies. In addition, unbiased molecular queries are showing promise in identifying signatures that correspond to prognosis and/or therapeutic outcomes. For example, in some cases, unique transcriptome signatures stratify cancers into distinct therapeutic and/or prognostic categories and thus improve patient management (e.g., Garraway, 2013). Thus far, this approach has been used primarily for determining which patients require aggressive chemotherapy treatment, hence reducing the frequency of over-treatment. Oncotype DX and FDA-approved MammaPrint tests, both based on distinguishing transcriptome signatures, are now utilized in the clinic to identify the low risk breast cancer patients to be excluded from aggressive treatment.

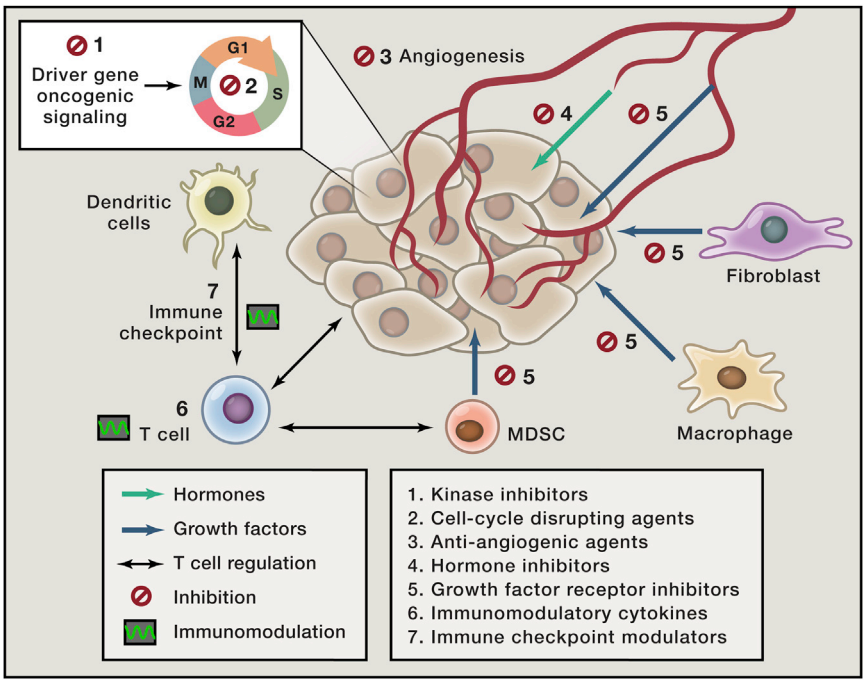

Figure 1. Targeting the Tumor and Its Microenvironment

Genetic alterations produce oncogenes that drive signaling pathways in cancer cells facilitating survival and growth. However, tumor cells also cooperate with stromal cells, including vessels, fibroblasts, and various immune cells, to acquire growth factors, an energy supply and protection from host defenses. These key autonomous and stromal mechanisms constitute potential therapeutic targets both locally, and for immune cells also in the circulating blood and distant immune organs, as shown by indicated numbers. (1) Cancer cell growth driven by an aberrant kinase ("Driver Gene") can be targeted by small-molecule inhibitors. (2) Oncogenic signaling promoting uncontrolled cell cycling can be disrupted (e.g., antimetabolites, anti-microtubule agents, DNA-damaging agents). (3) Tumor growth requires development of new vasculature for enhanced nutrient demands, which can be blocked by anti-angiogenic agents. (4 and 5) Growth of cancer cells stimulated by release of either host-derived hormones (4, green arrow) or growth factors (5, blue arrows from blood vessels, fibroblasts, macrophages, and myeloid-derived suppressor cells [MDSC]) can be targeted by hormone inhibitors (e.g., anti-hormones or biosynthesis inhibitors) or growth factor receptor inhibitors, respectively. (6 and 7) Tumor cells can shift the inflammatory response to an immunosuppressive mode (e.g., activation of CTLA-4 and PD-1 in T cells or PD-L1 in cancer cells). The immunosuppressive environment can be reversed via treatment of immunomodulatory cytokines (6, modulator sign; e.g., IL-2, IL-15) or immune checkpoint inhibitors (7, modulator sign; e.g., anti-CTLA-4, anti-PD-1, or anti-PD-L1), resuming anti-cancer activity of T cells. Left inset: key for therapeutic modes. Right inset: targeting agents. (Artwork adapted from design by Jonathan Marie).

Yet, accuracy is not optimal, and numerous challenges currently prevent broad implementation of molecular signature diagnostics (van't Veer and Bernards, 2008). Additionally, the hope is that molecular signatures can be identified via unbiased compound or molecular screens that will dictate specific effective treatments even when the targets are unknown.

Thus, although clearly impactful, the use of cancer molecular constitution to guide clinical practice is in its infancy, and research to identify parameters that hone specificity and improve accuracy is ongoing. If confined to human research, achieving maximum effectiveness is likely impossible due to low frequencies of each molecular subtype within most cancers and limitations associated with clinical trials. More challenging is understanding the impact of complex and varied inherited genetic constitution on clinical outcomes with subsequent conversion to clinical practice (Hood and Friend, 2011). In this regard, the sophistication of complex trait evaluation in mice using the collaborative and diversity crosses may offer a path to discovery (Churchill et al., 2004; Svenson et al., 2012).

The above summary provides only a cross-section of the therapeutic and diagnostic possibilities currently under investigation, and the reader is referred to current review articles for more comprehensive information (Chin et al., 2011; Hood and Friend, 2011; Yap et al., 2013). Ultimately, the current limitation to improving cancer patient care within reasonable timeframes may not be the availability of potentially efficacious therapies; rather, a major blockade is the lack of a fully developed and integrated set of reliable preclinical technologies that can navigate complex variables in therapeutic responses and diagnostic accuracy. To optimally develop efficacious therapies, preclinical research must utilize a diversity of models that collectively incorporate the biology and genetics dictating therapeutic outcomes for specific cancers, and yet achieve sufficient throughput. Here we summarize the value and constraints of mouse cancer models, highlight recent progress indicating promise, summarize non-mammalian and ex vivo preclinical models, and explore the needs for, and challenges to, developing robust multifaceted preclinical platforms for routine use.

Mouse Cancer Models in Preclinical Research

Murine cancer models designed to capture the complexities of human cancers currently offer the most advanced preclinical opportunity for navigating diverse mechanisms that provide rationale for therapeutic development (Van Dyke and Jacks, 2002). One approach is to probe pathobiology mechanisms to design effective treatments by perturbation with molecularly targeted therapies (Olive and Tuveson, 2006). Additionally, the models are being used/developed as preclinical efficacy determination platforms to guide clinical trial designs (Singh et al., 2012). However, the application of complex cancer models to clinical research directives is an emerging science, currently executed in individual settings and with limited resources. Significant research, ideally in a team-directed, multi-institutional effort, is required to hone existing technologies into integrated preclinical workflows to optimally accelerate positive clinical outcomes.

A variety of approaches to mouse cancer modeling are now available (Figure 2), and each has strengths and weaknesses (Table 1). Here, we address the limitations of standard Cell

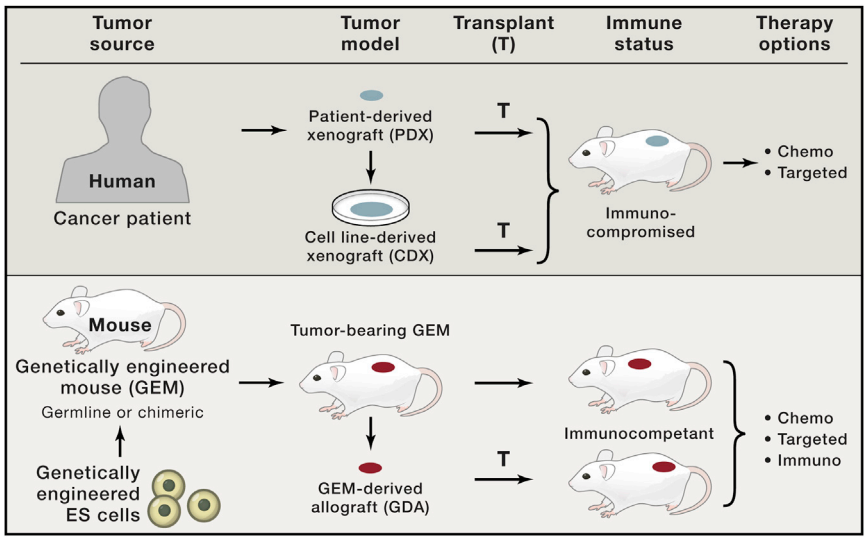

Figure 2. Current State of Preclinical Cancer Modeling

Preclinical mouse models can be defined according to the species source of tumor, how it is created, and how it is manipulated. (Upper panel) Tumors derived from human patients, and other non-murine species, can be directly transplanted into immunocompromised mice to form patient-derived xenograft (PDX) models; PDXs can also be established from circulating tumor cells (CTCs). Alternatively, these same tumors can produce established cell lines maintained in vitro as cell cultures, and transplanted into immunocompromised mice to form cell line-derived xenograft (CDX) models. Since the hosts of these tumors need to be immunocompromised, they are useful only for testing the efficacy of chemotherapeutics (Chemo) and targeted small-molecule inhibitors (Targeted). Xenograft models derived from canine patients also belong to this category, but are not shown here. (Lower panel) Mice can be engineered to generate tumors of human relevance with respect to histopathology, etiology, and molecular wiring. Offspring of such genetically engineered mice (GEM) can serve directly as preclinical models themselves, in which case the tumor is treated at its precise point of origin. Notably, model building can be streamlined by using non-germline approaches, one of which is to genetically modify ES cells and study the arising chimeric mice without time-consuming breeding schemes. Alternatively, tumors harvested from GEMs can be transplanted and expanded into fully immunocompetent syngeneic hosts, forming GEM-derived allograft (GDA) models. Syngeneic models allow preclinical studies of not only chemotherapeutic and small-molecule drugs, but also of all varieties of immunotherapeutic agents (Immuno).

line-Derived Xenograft (CDX) models, describe genetically and biologically engineered mouse cancer models [Genetically Engineered Mouse (GEM), GEM-Derived Allograft (GDA), Patient-Derived Xenograft (PDX) models], review values and constraints, and highlight recent progress. Thus far, results indicate promise in understanding cancer pathobiology and in the enhancement of clinical efficacy prediction, but also underscore the need for further development to achieve consistent reliability.

Traditional Mouse Models in Therapeutic Development

Historically, preclinical mouse models have co-evolved with cancer therapy development (Figure 3). The earliest models were built through transplantation of murine tumors into immunocompetent host mice (DeVita and Chu, 2008; Talmadge et al., 2007). These early mouse-in-mouse isograft models served as workhorses for drug screening during the 1960s and 1970s, and were successful in identifying a number of effective cytotoxic drugs such as vincristine and procarbazine (DeVita and Chu, 2008). During the 1980s, researchers explored mechanisms of metastasis using selected murine and human tumor cell lines. A series of investigations by Fidler and colleagues demonstrated that metastasis is not random but site-selective (Fidler and Hart, 1982), and that metastatic patterns are injection site-dependent, supporting the establishment of "orthotopic" models (Talmadge et al., 2007). Since then, cancer therapeutic development has relied upon the more tractable CDX transplantation models, in which tumors develop after subcutaneous injection of in vitro-established human cancer cells into immunocompromised mice (Figure 2). The cell lines have been selected over many passages for rapid 2D growth on plastic in serum-containing media. The NCI60 cell line panel (DeVita and Chu, 2008; Talmadge et al., 2007) provided a valuable resource from which most CDXs were generated, and recent efforts have greatly expanded the repertoire (Reinhold et al., 2015). These models are easily established in a wide variety of laboratory settings and have been successfully used to identify an abundance of cytotoxic drugs leading to chemotherapy treatments that still dominate clinical cancer management (Figure 3).

Unfortunately, CDXs have failed to predict human efficacy for most therapies targeted to cancer-driving proteins (Johnson et al., 2001), as evidenced by the low FDA approval rate of 5%–7% for targeted therapeutics (Sharpless and Depinho, 2006). With an average time from discovery to clinical practice of 12 years, at an average estimated cost of $0.5–$2.0 billion (Adams and Brantner, 2006) and an immeasurable human price, this low yield forestalls even a goal to chronically manage, rather than cure, cancers. The observation that most cancer therapeutics fail in clinical phase II and III efficacy assessment indicates that current standard preclinical practice inadequately addresses complex challenges to successful treatment, such as host immune responses, cancer heterogeneity, and drug resistance. Consequently, the system cannot be used to optimize a multitude of variables known to influence therapeutic outcomes, such as combinatorial therapies, dosing schedules, and drug delivery methods (Al-Lazikani et al., 2012). CDXs continue to be valuable in identifying non-targeted cytotoxic agents and in primary assessment of drug toxicity (Teicher, 2006), for analyzing resistance mechanisms (Garraway and Jänne, 2012) and in triaging potentially effective targeted therapies for evaluation in more representative models.

Mouse Models Designed after Patient Cancers

Mice and humans are believed to have diverged from each other ~87 million years ago (Bailey et al., 2013), so naturally there are numerous significant similarities between the two species, and also many marked disparities, including differences in immune systems and drug metabolism. Based on the premise that many cancers have been cured in mice and not in people, many argue

Table 1. Comparison of Clinical and Preclinical Model Properties

	Immune Status of the Host	Micro-environment Context	Human Relevance	Tissue Availability	Disease Complexity	Experimental Robustness	Initiation/ Progression	Feasibility in Pathway Engineering	Cost
Clinical Trials	Functional	Natural	Standard	Highly Limited	High	Low	Intact	Irrelevant	Very High
Cancer Cell Line-Derived Xenografts (CDX)	Deficient	Xenogeneic	Situational* (passage number dependent)	Expandable	Low	High	Bypassed	Yes	Low
Patient cancer-Derived Xenografts (PDX)	Deficient	Xenogeneic	High	Expandable/ Limited	Moderate	High	Bypassed	No	High
Genetically Engineered Mice (GEM)	Functional	Natural	High/ Variable (model dependent)	Limited	High	Moderate/ Variable	Intact	Yes	High
GEM-Derived Allografts (GDA)	Functional	Allogeneic	Low/ Variable	Expandable	Moderate	High	Bypassed	Yes	Moderate

*CDX models have been shown to be limited as predictors of clinical outcome. However, they continue to be valuable for evaluating resistance mechanisms, for identifying non-targeted cytotoxic agents, for assessing drug toxicity, and as a platform to triage potentially effective targeted therapies. In general, the longer cells are in culture, the further they drift from normal tumor evolution, and the less relevant they become.

that mice are inappropriate for use in therapeutic development (Leaf, 2004). However, it is critical to understand that "cures" have been attained only in CDX models, thus dismissal of *all* mouse cancer models as irrelevant is unwarranted. Human cancers are enormously complex, and their evolutionary etiology generates vast diversity among and within them, thus challenging the attainment of successful treatments. However, as knowledge of cancer complexities has increased, so has the ability to design mouse models that better represent cancer patients. PDX and GEM models develop tumors with the greatest similarity to human diseases yet achieved, and the past 5 years have seen an increase in their employment in preclinical research. As with all models, each approach has its strengths and limitations (Table 1). Early studies suggest promise for improved guidance in the development of successful clinical treatments (Table 2), and yet also emphasize the need for further scrutiny and refinement. The following provides a balanced consideration of model advantages and limitations, their ramifications in obtaining optimally accurate preclinical data, and the logistical requirements for achieving efficiency, accuracy, and reproducibility.

Patient-Derived Xenograft Models

Relative to CDX models, immunocompromised mice bearing subcutaneous surgically derived clinical tumor samples (PDX models) are better aligned with human disease, since intact tissue that preserves tumor architecture is transferred directly to recipient mice and not compromised by in vitro adaptation (Figure 2). PDXs are the only models harboring bona fide tumor targets directly from the patient, and hence their use in drug discovery is expanding rapidly. Promise for such models, first developed by Fiebig (Fiebig et al., 1984), was demonstrated when chemotherapeutic agents, such as alkaloids and anti-metabolites, were shown to elicit similar responses in mice and patients (Mattern et al., 1988). In contrast, a study of responses to numerous cytotoxic agents in NCI60-based CDX models showed that the predictive value for efficacy was much less impressive (Johnson et al., 2001). Unfortunately, early studies utilizing PDXs were limited by difficulties in collecting clinical samples and in achieving sufficient take rates.

The recent resurgence of PDX model use for therapeutic evaluation has been fueled by significant improvements in clinical sample access and transplantation technology. Cancers established as PDXs can, in early passages, retain the stromal composition and histologic and molecular heterogeneity characteristic of those in patients (Hidalgo et al., 2014; Tentler et al., 2012). Since these properties critically impact therapeutic responses and biomarker specificity, PDX models provide a preclinical venue for addressing some of the most challenging barriers to successful patient therapy. Furthermore, human target specificity allows for direct evaluation of lead human-specific therapeutics, such as antibodies, in clinical development.

Methodologies for PDX establishment and characterization are detailed elsewhere (Hidalgo et al., 2014; Tentler et al., 2012; Zhang et al., 2013). For some cancers, such as certain melanomas, lung, and colorectal cancers, transplant take rates can reach $\geq$75%, and the time required for tumor growth can be as little as 2–4 months. However, these attributes vary widely depending on sample type and amount (e.g., fresh biopsy tissue, fine needle aspirate, circulating tumor cells), tumor origin, molecular properties, and recipient strain (see Supplemental Information).

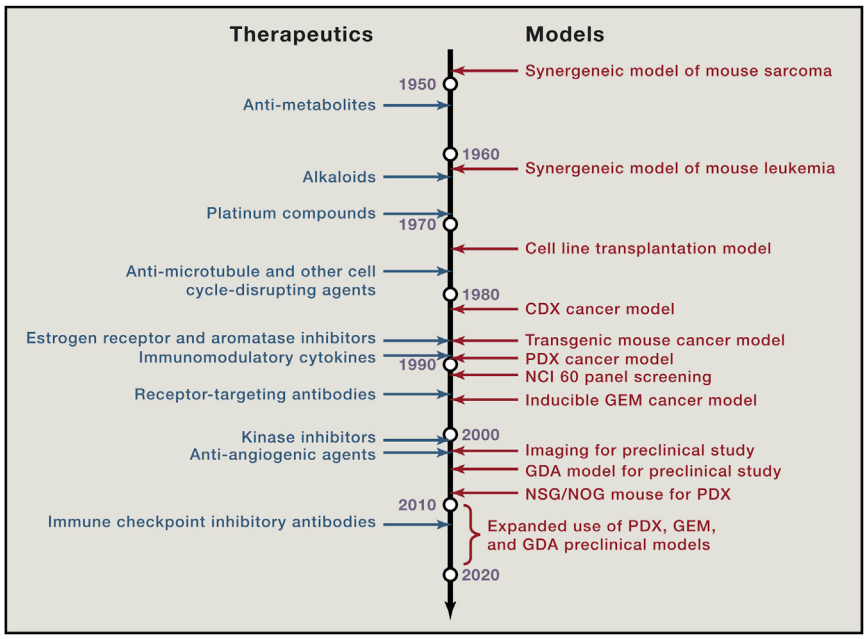

Figure 3. Timeline of Key Preclinical Cancer Model Developments since 1950

As the conceptual targets of cancer treatment progressed from actively dividing cells to oncogenic signaling and immune checkpoints, preclinical models (right side) and cancer therapies (left side) co-evolved accordingly. This evolution was highly dependent on technical advances, resulting in waves of activity. For example, recent development of fully immunocompromised mice and diverse syngeneic GEM models has significantly promoted PDX and GDA models, respectively, for preclinical cancer studies (the bracket).

Consequently, some cancers, such as neuroendocrine, luminal ER+ breast, and prostate cancers (Rosfjord et al., 2014) are underrepresented. Notably, PDX engraftibility appears to significantly correlate with clinical aggressiveness (Ilie et al., 2015).

Relative to subcutaneous transplants, cancers orthotopically transferred into organs of origin are more likely to maintain tumor microenvironment characteristics that impact therapeutic outcomes (Talmadge et al., 2007). However, orthotopic PDX production is technically challenging, and, for most cancer types, tumor growth and responses must be monitored via expensive and often laborious longitudinal imaging. Thus, preclinical therapeutic studies currently exclusively utilize subcutaneous models.

Production of PDX cohorts is by serial tumor transplantation, and, given the likelihood of change with each passage, therapeutic studies are most representative in low-passage models. Additionally, human stromal components are maintained for only 2–3 passages, with mouse stromal elements becoming dominant thereafter (Rosfjord et al., 2014). Unfortunately, if limited to early passage use, each model represents a limited resource. Hence, most preclinical studies utilize models that have been expanded, banked, and developed into significantly sized cohorts. The extent of sacrifice in accurately predicting efficacy is presently undefined and likely depends on the mechanism of therapeutic activity. As such, in propagating PDXs, parental tumor traits should be routinely monitored, and deviations must be considered in interpreting therapeutic and biomarker data.

To circumvent immune rejection, human cancers must be transplanted into immunocompromised mice. Commonly used recipients, such as nude, SCID, and NOD/SCID strains, vary in the extent of immune impairment (detailed in Supplemental Information). IL-2Rγ-deficient NOD/SCID mice (NSG and NOG strains) are the most severely impaired, and often yield improved take rates. Critically, the requirement for immunocompromised hosts precludes assessment of arising therapies designed to modulate immune function (e.g., immune checkpoint inhibitors α-CTLA-4, α-PD-1, α-PD-L1). Moreover, therapeutic responses in general are likely influenced by preexisting cancer-dependent immune phenotypes and immune responses elicited upon therapy-induced tumor perturbation (Zitvogel et al., 2008). The extent to which compromised immune systems limit predictive value for a given therapeutic approach will be determined as comparisons between PDX and clinical outcomes are expanded. Technologies to "humanize" the mouse immune system by transplanting purified human CD34$^+$ hematopoietic stem cells into myeloablated NSG/NOG recipients (e.g., "BLT" mice: http://jaxservices.jax.org/invivo/humanized-BLT-mice.html) and other chimeric strategies have been developed (Legrand et al., 2009; Shultz et al., 2014). However, the high cost of recipient mice, limitations on human bone marrow acquisition, engraftment variability, and technical demands currently preclude use of these models in preclinical therapeutic discovery.

Despite the challenges to routine preclinical application, several PDX studies have proven effective in paralleling human outcomes (Malaney et al., 2014), in exploring drug resistance mechanisms (Das Thakur et al., 2013) and in identifying targets for second-line treatment (Girotti et al., 2015). Programs are also underway to employ PDX models in individualized precision cancer care. To date, this approach has been most successfully applied to pediatric patients with advanced sarcomas who have demonstrated the predicted response, sometimes to drugs not previously associated with this indication (Tentler et al., 2012). Patient-specific studies are currently limited by expense and relatively long and unpredictable times for establishing test animals. Since current clinical trials generally involve patients who have undergone prior failed treatments, results may not always be obtainable in a beneficial timeframe.

Genetically Engineered Mouse Cancer Models

Of all murine cancer models, GEMs provide the most complete representation of cancer development; cancers develop from initiation through progression, co-evolve with intrinsic stroma, and possess an intact immune system (Figures 1 and 2). However, GEM models are the most challenging to work with effectively, and species differences must be carefully considered in

Table 2. Representative Clinically Relevant Mouse Trials

Trial Design	Cancer Type	Model Type	Engineered Drivers	Drugs/ Treatment	Significance	Relevant Publications
Preclinical	Hematopoietic (APL)	GEM	PML-RARα fusion PLZF-RARα fusion	Retinoic acid	Demonstrated the efficacy of retinoic acid plus As_2O_3 in specific APL subtypes, validated in clinic	(Ablain and de Thé, 2014; Pandolfi, 2001)
Preclinical	Pancreas (Neuro-endocrine)	GEM	RIP1-Tag2	Sunitinib	Demonstrated the efficacy of Sunitinib plus Imatinib, validated in clinic. FDA approved for pancreatic cancer treatment in 2011.	(Pietras and Hanahan, 2005; Raymond et al., 2011)
Preclinical	Medulla-blastoma	GEM	$Ptc1^{+/-}$ $P53^{-/-}$	GDC-0449 (SMO inhibitor)	Demonstrated the efficacy of an Shh pathway small molecule inhibitor, validated in clinic	(Romer et al., 2004; Rudin et al., 2009)
Preclinical	Pancreas (Neuro-endocrine)	GEM	RIP1-Tag2	Erlotinib Rapamycin	Demonstrated efficacy of combining drugs targeting EGFR and mTOR	(Chiu et al., 2010)
Co-clinical	Pancreas (PDA)	GEM	LSL-$Kras^{G12D}$ LSL-$Trp53^{R172H}$ Pdx-1-Cre	Gemcitabine Nab-Paclitaxel	Provided mechanistic insight into clinical cooperation between Gemcitabine and Nab-Paclitaxel	(Frese et al., 2012; Goldstein et al., 2015)
Co-clinical	Pancreas (PDA)	GEM	LSL-$Kras^{G12D}$ LSL-$Trp53^{R172H}$ Pdx-1-Cre	CD40 monoclonal antibody Gemcitabine	Demonstrated that targeting stroma was effective in treatment of metastatic PDA	(Beatty et al., 2013)
Co-clinical	Lung (NSCLC)	GEM	$KRAS^{G12D}$ $p53^{fl/fl}$ $Lkb1^{fl/fl}$	Selumetinib Docetaxel	Validation of improved response of adding Selumetinib to Docetaxel treatment	(Chen et al., 2012; Jänne et al., 2013)
Co-clinical	Lung (NSCLC)	GEM	EML4-ALK fusion	Crizotinib Docetaxel Pemetrexed	GEM model predicted clinical outcome of drug combinations	(Chen et al., 2014; Lunardi and Pandolfi, 2015)
Co-clinical	Various Sarcomas	PDX	N/A	Various chemotherapies	PDX testing predicted clinical outcome of drug combinations	(Stebbing et al., 2014)
Postclinical	Ovarian (SEOC)	GDA; PDX	RB/p53-deficient BRCA1/2-deficient	Olaparib Cisplatin	Validation of treatment efficacy in BRCA mutant tumors in both GDA *and* PDX models	(Kortmann et al., 2011; Szabova et al., 2014)
Postclinical	Pancreas (Neuro-endocrine)	GDA	RIP1-Tag2	Anti-VEGFR1 and anti-VEGFR2 antibodies	Identification of mechanisms of resistance to anti-angiogenic therapies	(Casanovas et al., 2005)
Biomarker	Lung (NSCLC)	GEM; Carcinogen-induced	Various Models	N/A	Used in-depth quantitative MS-based proteomics to profile plasma proteins	(Hanash and Taguchi, 2011)
Biomarker	Pancreas (PDA)	GEM	$Kras^{G12D}$ $Ink4a/Arf^{fl/fl}$ Pdx-1-Cre	N/A	Used in-depth proteomic analyses to identify candidate markers applicable to human cancer	(Faca et al., 2008)

experimental designs and interpretations. Extensive experience and infrastructure are required to ensure the use of optimally accurate models and to achieve sufficiently populated well-controlled preclinical studies. Yet, GEM cancer models provide the only opportunity to evaluate drug delivery, therapeutic response, and biomarker expression for cancers evolving within their natural microenvironment (autochthonous cancers). These complex dynamic processes contribute to overall disease properties, and in particular, constitute a source of the inter- and intra-tumoral heterogeneity that challenges successful therapeutic development. Additionally, the accuracy of some therapeutic interventions, such as those targeting the immune system, may depend on the constitution of evolutionary, rather than transplanted, disease. Indeed, overall, GEMs and GEM-derived models are currently the *only* preclinical platform for evaluation and optimization of immunomodulatory therapies. Although some immune properties differ in mouse and human, there is significant conservation (Bailey et al., 2013); moreover, many differences can be managed via data interpretation or minimized by using genetically engineered "humanized" models (Scheer et al., 2013). Finally, autochthonous GEMs are the only viable models for evaluating prevention therapies.

Several reports show that well-designed GEM studies can contribute to improved clinical trials (Table 2), not only in identifying potentially efficacious therapies but also in predicting both positive and detrimental effects in molecular subclasses. A major power of GEM approaches is in the flexibility to create models with precise molecular specificity. With increasing sophistication, several strategies (summarized below and detailed elsewhere [Abate-Shen et al., 2014]) have been employed over the past three decades to significantly enrich our understanding of cancer mechanisms. A plethora of genes frequently altered in human cancers have been validated as disease drivers in GEMs, thereby facilitating the evaluation of cancer evolutionary mechanisms and kinetics, susceptible cell and molecular targets, relative cancer cell and microenvironment roles, and mechanisms of invasion and metastasis. Indeed, entire natural disease histories can be mapped (Stiedl et al., 2015; Van Dyke and Jacks, 2002).

In the process of basic discovery, countless GEM cancer models representing a variety of histiocytic cancer types driven by multiple independent drivers have been produced, and many are currently used in preclinical evaluations. Although *no model* can perfectly capture the human condition, several GEM models tractable for preclinical studies develop cancers with remarkable molecular and pathologic similarity to their human counterparts. However, since most established GEMs were created to address basic mechanisms, many do not accurately model human disease and/or are intractable for effective preclinical evaluation. Furthermore, each engineering approach can elicit untoward anomalies. Such circumstances can be accommodated in the interpretation of mechanistic studies, but are the basis for exclusion of many models for effective preclinical research. Thus, choosing appropriate models as subjects for preclinical discovery requires a deep understanding of cancer biology and genetics *and also* of engineering modalities. The following provides a reasonable guide for optimizing the value of GEM-based preclinical platforms.

Germline GEMs

An extensive array of technologies is employed to engineer the mouse germline with great precision. By editing the genome of embryonic stem (ES) cells or zygotes, mice can be programmed for cell-type-specific disruption of tumor suppressor genes via direct mutation or expression of interfering non-coding RNAs (RNAi) (Walrath et al., 2010) and for oncogene expression at physiological or cancer-analogous levels. Furthermore, mice can be "humanized" by engineering the expression of drug targets in relevant cell types (Scheer et al., 2013) so that human-specific targeted therapies, such as antibody-based drugs, can be tested in GEM models. While traditional methods for constructing locus-specific genetic changes require significant lead times for engineering, the recent development of rapid sequence-targeted approaches (Mou et al., 2015) has significantly reduced this time to weeks instead of many months. In particular, clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 technology, which is efficient and versatile, is accelerating germline engineering and also facilitating rapid somatic engineering (see below).

Depending on the strategy, expression of an engineered "event" can be constitutive or inducible, although gene induction with cell-type and temporal specificity provides the best possibility for accurately modeling disease development. Inducibility is achieved by combining cell-specific expression of transcription factors (e.g., doxycycline-modulated tet-transactivators) or recombinases (Cre-*lox* or Flp-*FRT*) with cognate *cis* elements linked to a target gene, or by expressing proteins fused with a hormone-responsive domain (e.g., the tamoxifen-inducible estrogen receptor domain) (see Supplemental Information). When multiple distinct inducible systems are combined within the same cancer model, cancer-specific mutations can be induced sequentially in order to map and emulate cancer evolution (e.g., [Young et al., 2011]) and thus to generate increasingly relevant preclinical models. Reversible inducibility can be achieved with each of these technologies, although small molecule-mediated modulation of transcription factors and hormone-responsive domains are the most tractable for toggling expression on and off (Abate-Shen et al., 2014; Texidó, 2013). This approach facilitates the identification of events required to sustain tumor growth ("oncogene addiction") (e.g., Soucek et al., 2008) and thus of potential therapeutic targets (e.g., Kwong et al., 2012). Tumor responses to the shutdown of oncogenes or restoration of functional tumor suppressors within tumors, or appropriate effector cells, indicate the potential efficacy of targeted therapies, while genetic ablation in the entire animal predicts the overall toxic effects of specific inhibitors. However, since off-target effects will not register in this approach, results only indicate whether a given therapy is *potentially* efficacious.

A critical, often overlooked, consideration when building GEM cancer models is the incorporation of known environmental etiologies. However, there are notable examples wherein certain environmental factors were validated as etiologic agents and thus produced representative cancer models, including HPV E6/E7-induced cervical cancer (Riley et al., 2003), UV accelerated melanomas (BRAFV600E [Cao et al., 2013], mutant HRAS [Kannan et al., 2003], and HGF/MET [Noonan et al.,

2001] models), and *Helicobacter*-fueled gastrointestinal cancers (Rogers and Fox, 2004). Exposure of GEM cancer models to environmental mutagens can be used to approximate the mutation load of many human cancers (e.g., Westcott et al., 2015), which influences therapeutic outcomes such as in drug-resistant relapse and neoantigen load-dependent immunomodulation.

The extent to which findings in GEMs extend to patients depends on engineering mice based on our understanding of human cancer etiologic drivers, cellular origins, heterogeneity, pathogenesis, and clinical properties. To recapitulate human cancer development, clinically relevant driver gene(s) or pathways must be perturbed in relevant target cells. For adult cancers, gene expression should be targeted to adult, rather than developing, organs. Furthermore, for optimal modeling, cancers should progress in a relevant sequence, since the order of events impacts properties of evolving tumors. Ideally, both initiation and progression to aggressive cancer should be evaluated using individual and relevant combinations of molecular aberrations thought to be causal in humans. High phenotypic penetrance and consistency among animals within a lineage are essential for tractability.

The accuracy of disease modeling depends on *actually achieving* the specificity envisioned in experimental designs, which is not always realized because of technical limitations and/or gaps in current knowledge. Of course, engineered sequences must be validated, but it is also critical that expected transcriptional specificities be confirmed. Unless targeted to specific genomic locations, transgenes insert randomly, and expression can be dramatically altered depending on insertion sites. Furthermore, transgenes may not carry all necessary regulatory signals. Hence, several founder lines should be established and fully characterized before selecting accurate representative lines for modeling cancers. Even targeted genetic changes have the potential to alter gene regulation. Thus, specificity, levels, and range of expression must be evaluated for each model; aberrant expression usually alters disease and can also yield ectopic phenotypes that hinder tractability and invalidate data. Yet, a surprising number of existing engineered strains, including those driving inducible expression, are not fully characterized. Hence, when choosing cancer models for preclinical studies, it is essential that expression and disease patterns are well established and accurately represented (see Supplemental Information).

Non-Germline GEM Models

While autochthonous GEM models have great utility, most are not tractable for large-scale screening of multiple anti-cancer drug candidates due to high cost, long timelines, extensive complex breeding, and/or difficulties in obtaining synchronous tumorigenesis. Preclinical analysis of metastatic lesions is particularly challenging; primary tumors arise stochastically with no reliable timetable, as in humans, and multiple tumors often develop. Thus, extensive longitudinal tomographic imaging is required to enroll mice bearing similarly sized tumors for therapeutic evaluation (e.g., Weaver et al., 2012). Such procedures require specialized expertise and can be too expensive and time-consuming for first-line drug screening. Several strategies to produce ''non-germline'' GEMs have been developed that bypass breeding, reduce expense, and, in some cases, improve flexibility, uniformity, and timelines (Heyer et al., 2010).

GEM-Derived Allograft Models. GEM-derived allograft (GDA) models marry the genetic and biologic human cancer similarities of GEM models with the relative ease of transplantation technology of PDXs (Heyer et al., 2010). Without in vitro manipulation, tissue fragments derived from tailor-made GEM tumors are expanded by transplantation, orthotopically or subcutaneously, into immunocompetent syngeneic hosts (Figure 2). Thus, tumors can be banked to facilitate large cohort production, and efficacy studies can be performed in industry-friendly timeframes (~3–8 weeks), allowing for increased throughput. Indeed, a battery of treatments can be evaluated in GDAs prior to (preclinical) or parallel with (''co-clinical'') clinical trials (Table 2). As with GEMs, immune systems are fully functional in GDAs, and interactions among tumor cells and their intrinsic microenvironments are maintained.

GDAs are particularly amenable to the evaluation of metastatic disease, which is responsible for most cancer-related deaths and is rarely assessed preclinically. In GDAs, metastases occur from *single* primary tumors, which can be resected to allow time for metastatic progression (Day et al., 2012). This approach also emulates clinical care standards for many cancers and facilitates comparing therapeutic responses of both primary and metastatic disease derived from the same GEM cancer (Figure 4).

As with PDXs, serial passaging increases the likelihood that tumor properties will deviate from parental samples due to further evolution and/or selection of sub-compartment growth; thus, transplanted tumors should be monitored for molecular and biological similarity to founding tumors. Additionally, since transplanted tumors do not evolve in situ, GDAs cannot legitimately be used for prevention studies, and some therapeutic outcomes may differ between autochthonous GEMs and GDAs. Given the potential tradeoff of accuracy for tractability, candidate therapies efficacious in GDAs should be subsequently validated in the original GEM models prior to clinical studies.

Stem Cell-Derived Chimeras and Somatic Models. Mice chimeric for genetically engineered cells are created through implantation of GEM-derived or genetically manipulated ES cells into pre-implantation embryos. Since oncogenic alleles are engineered ex vivo in ES cells, many mice with the desired genetic composition can be generated in the absence of complex, laborious, and long-term breeding schemes. The potential value of this approach was first highlighted in the production, analysis, and preclinical evaluation of lung adenocarcinoma (Zhou et al., 2010). Once constructed, ES cells harboring the desired alleles can be derived from blastocysts produced by a penultimate cross. In turn, this bankable resource can be used to generate mice chimeric for mutant and wild-type cells (Premsrirut et al., 2011), facilitating conditional RNAi-mediated knockdown of target expression via manipulation of ES cells, which can then be used to generate chimeric mouse cohorts (Dow et al., 2012).

Notably, the advent of CRISPR/Cas9 technology, and with it the ability to perform complex gene editing with relative ease and speed, has dramatically enhanced the value of non-germline GEM approaches. Several groups have precisely modified oncogenes and tumor suppressor genes directly in somatic cells of adult mice, significantly improving the feasibility and flexibility of this genetic engineering approach (Chen et al., 2015; Dow

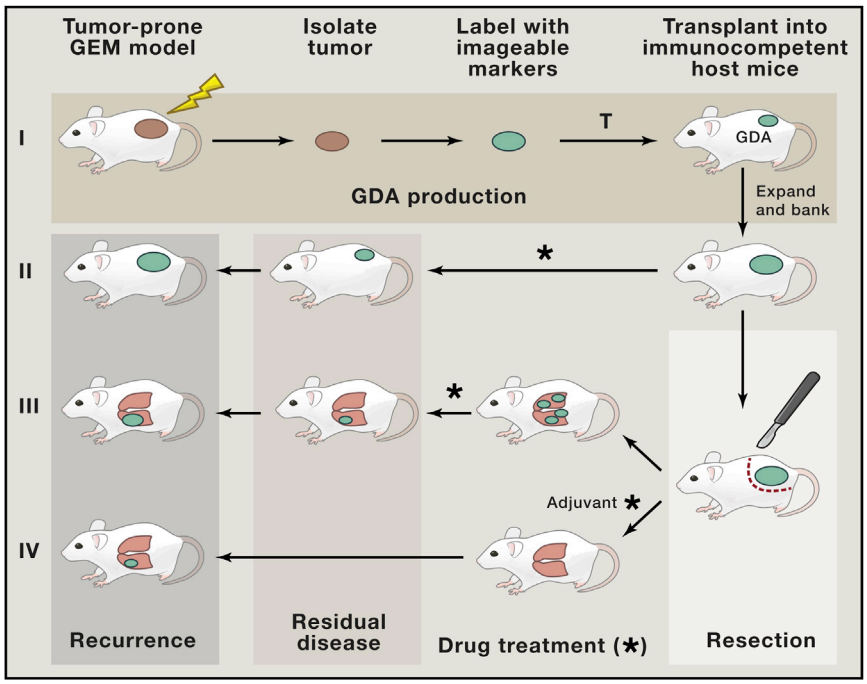

Figure 4. Generation and Application of Metastatic GDA Models

(I) GDAs are derived from tumors arising in mice genetically tailored to produce human-relevant models. Relevance can be further enhanced by including appropriate etiological agents (lighting bolt). Arising tumors are resected, labeled with imageable markers (green), and directly transplanted into fully immunocompetent syngeneic mice at either subcutaneous or orthotopic sites. The imageable markers allow monitoring of tumor growth and drug response, and/or FACS purification for analysis. Once successfully transplanted, GDAs can be expanded for banking and/or preclinical studies. Mice bearing GDAs can be treated directly with individual or combination drugs (*) to study therapeutic efficacy at the "primary" tumor site (II). (III and IV) Alternatively, GDAs can be resected using survival surgery, and treatments focused on metastatic disease, simulating first-line treatment in human patients following primary tumor resection. GDA models allow for interventive treatment of metastatic disease once detected (III), or preventive adjuvant treatment initiated immediately following surgical resection (IV). GDA models are thus well suiting for studying primary or metastatic disease, with interventive or preventive approaches using pathway-targeted small molecule and/or immunotherapeutic agents.

et al., 2015; Maddalo et al., 2014; Platt et al., 2014). These models also better mimic human cancer relative to standard germline GEMs in that tumors typically arise from fewer cells in the context of normal stroma

In a variation of the non-germline GEM approach, genetically engineered stem or progenitor cells can be transplanted into syngeneic mice, where they can home to appropriate tissue targets and become the cells of origin for developing tumors (Heyer et al., 2010). These models are especially amenable for studying hematopoietic cancers, where the stem cells are well characterized and the host can be prepared for receiving transplanted cells by using irradiation to create a favorable niche for the engineered hematopoietic stem/progenitor cells to colonize. Successes have also been reported for other cancers (Heyer et al., 2010).

Logistics for Optimizing Preclinical Studies

Extensive complexities that impede successful drug development in cancer patients dictate that faithful murine cancer models must themselves be complex. Both PDX- and GEM-based models offer this opportunity. However, their very complexity warrants that informative models are generated and characterized with substantial knowledge of cancer mechanisms and modeling limitations, rigorous animal maintenance and production, routine phenotypic and genetic monitoring, appropriate strategies for therapeutic response evaluation, and consideration of multiple variables that impact data interpretation. To achieve routine therapeutic and biomarker development that positively influence patient care, preclinical studies must be (1) well-powered with significant cohort sizes and several evaluation parameters, (2) goal-oriented and efficiently executed, and (3) highly reproducible.

Experimental Considerations

Once models that optimally represent human disease have been selected, clinical relevance relies on experimental parameters that are comparable and/or translatable to human practice. These include, but are not limited to, dosing levels and schedules, drug pharmacology, response evaluation methods, and endpoint choices. Therapeutic agents' pharmacokinetics (PK) and ability to modify targets when known (pharmacodynamics; PD) should be measured in tumor-bearing mice. The fate of administered drugs is largely determined by drug metabolizing enzymes essential for their absorption, distribution, metabolism, and excretion (ADME). Therefore, the differences that exist between the central metabolizing enzymes in mice and humans, the cytochrome P450 (CYP) family, constitute a confounding factor in extrapolating drug PKs and the responses they elicit. Since the maximum tolerated dose (MTD) of many drugs in mice is significantly higher than in humans, it is essential to evaluate efficacy by using doses achievable in patients. However, this is possible only when human PKs are known; for example, for repurposing FDA-approved drugs, for preclinical evaluation of combination therapies that comprise single phase II agents, and for co-clinical experimentation wherein mouse and human evaluations are performed in parallel, such that clinical toxicity results are available. Even when appropriate human dosing is known, there is no simple formula for approximating comparable doses to achieve the same PK in mice, and instead experimental determination is required (Sparreboom et al., 1996). Yet, when evaluating numerous agents, this approach is not possible; rather, subsequent coordination of clinical results and further preclinical dose escalation experiments are needed for optimal response assessment.

In an effort to apply a genetic solution to the PK problem, a number of humanized CYP GEMs have been developed (Gonzalez, 2004; Scheer and Wolf, 2014). Despite these advances, the humanized alleles have not yet been incorporated into GEM cancer models or PDX recipients. Such an undertaking will require significant resources, substantial time, and community effort to generate and evaluate revised models. Nonetheless, the investment will be worthwhile if the gap between laboratory mice and patients is narrowed.

The choice of preclinical experimental endpoints to determine therapeutic responses is also critical for achieving outcomes most representative of those in patients (Talmadge et al., 2007). In prevention studies, efficacy is based on disease-free or minimized status. For intervention therapy, efficacy is justified by overall survival and should not be judged solely on tumor growth inhibition. The importance of survival endpoints is highlighted by a pancreatic cancer clinical trial designed based on short-term GEM studies demonstrating reduced tumor volumes in response to sonic hedgehog pathway inhibition combined with gemcitabine (standard of care) compared to gemcitabine alone (Olive et al., 2009). Unfortunately, the trial terminated early due to increased disease dissemination and poor patient survival. However, subsequent survival studies in the GEM model replicated the clinical result, demonstrating that initial drug effects did not predict survival outcomes. Hence, the model appropriately predicted patient responses, but only with a meaningful endpoint (Couzin-Frankel, 2014).

Tumor growth and therapeutic responses in subcutaneous transplant models, such as CDXs, PDXs, and GDAs can be monitored by standard caliper measurement. Tumor growth in autochthonous and orthotopic transplant models (other than skin and breast models) and in all metastatic models must be monitored by longitudinal imaging strategies (Wang et al., 2015). High-resolution 3D images are compiled from sectional images generated by tomographic scanning of signals from X-ray (CAT), magnetic field-excited atoms (MRI), or injected radioactive tracers (SPECT; PET) (Supplemental Information). Tomographic imaging requires specific expertise for accurate execution and is relatively expensive and time-consuming. Optical imaging, which detects visualized wavelengths generated from excited fluorescent chromophores (e.g., jellyfish GFP) or firefly luminescent reactions (e.g., luciferase), can be employed for detection in real-time and is cost- and time-effective; however, these methods do not produce accurate tomographic data and are limited by tissue absorption. Notably, traceable marker proteins required for optical imaging are xenogeneic with respect to mammals and can induce immune responses in immunocompetent mice, which can result in inconsistent activity, graft rejection and/or inhibition of metastasis, confounding data interpretation. Hence, effective employment of xenogeneic reporters is restricted to short-term studies or studies in immunocompromised models, limiting their usefulness in preclinical science (Steinbauer et al., 2003). However, this problem can be circumvented, at least in part, by employing host mice genetically engineered to express respective markers at an early age, which elicits tolerance and thus recognition as ''self'' (Day et al., 2014).

Several additional points associated with preclinical trial design are worth emphasizing. Tumor mass is a critical factor in preclinical studies; vastly different outcomes can result from initiating drug dosing when tumors are different sizes. Moreover, human tumors are typically much larger than their mouse counterparts, which could affect how preclinical data translates to the clinic. It is also vital to run preclinical trials with a sufficient number of animals in each experimental arm to achieve statistically significant results; ensuring statistical power must be considered a priority for any preclinical study. Therefore, it is prudent to consult biostatisticians prior to finalizing study designs. Finally, the influence of genetic background on tumor behavior can be significant, and must be considered when designing model systems. Generation and analysis of mouse cancer models within the collaborative cross, a large panel of inbred mouse strains (Churchill et al., 2004), could also provide important insights into the impact of complex germline genetics on tumor predisposition and drug response.

Infrastructure

Critical work establishing the utility of murine cancer models in preclinical research has taken place in independent laboratories over the last 20 years. However, because of severe resource limitations, the absolute need to perpetuate basic investigator-driven mechanistic discovery, and an increasingly competitive environment wherein success is measured by individual merit, the opportunity for laboratories to execute preclinical studies beyond the pilot level is limited. Recent reports indicate that most preclinical outcomes at this level are not reproduced when studies are not conducted with robust experimental standards, such as inclusion of appropriate positive and negative controls, execution with sufficient statistical power, attention to pharmacological considerations, and implementation of blind evaluations (Begley and Ellis, 2012; Begley and Ioannidis, 2015). Adherence to *all* these standards is simply not possible in individual laboratories under current conditions. To increase accessibility to preclinical evaluation in murine cancer models, several institutions have established core facilities that perform studies using dedicated staff and common methodologies. These cores represent a necessary step to improve reproducibility in preclinical outcomes. Yet, most core facilities do not have the resources to instate the full range of skills and technologies indicated in ''*Experimental Considerations*'' above to ensure optimal quality and replication of clinical approaches. Additionally, conducting well-powered blinded studies requires a sizable dedicated staff, which is generally not achievable in academic cores. Finally, global improvement of murine preclinical research must include the generation of an increased range of well-characterized, technically tractable and optimally accurate models vetted for preclinical evaluation, along with the development of exportable standard operating procedures (SOPs).

To address these needs, over the past decade several organized efforts have been established that are dedicated to: (1) improving the accuracy and reproducibility of preclinical drug development platforms; (2) developing and exporting SOPs and models; (3) understanding cancer pathobiology through targeted therapeutics; and/or (4) applying the outcomes of optimized preclinical therapeutic and biomarker studies to clinical research for improved patient care. Common attributes in each

Table 3. Future Challenges and Possible Solutions for Mouse Preclinical Cancer Trials

Issue	Challenges	Possible Solutions
Model improvement	More precise spatial and temporal control of genetic alterations in mouse tissues	Improve technologies for genomic editing (*e.g.,* CRISPR) and regulating gene activity
	Human relevance of stroma, immune system, and therapeutic targets in mouse cancer models	"Humanize" genes via genetic engineering and immune system by reconstitution with human hematopoietic stem cells
	Recapitulation of the tumor heterogeneity found in human cancers	Introduce environmental etiological factors (e.g., UV in skin cancer models); allow tumor evolution by avoiding inappropriately dominant oncogenic drivers
Study setting	Difficulties in diagnosis and treatment of large cohorts of mice as individual patients	Synchronize tumorigenesis by adopting inducible GEM or transplantable GDA systems
	Disease progression and clinically relevant endpoints in preclinical study	Improve biomarkers and imaging techniques for tumor tracking; adopt clinically relevant endpoints (e.g., progression-free survival)
	Integration of pathologic, genomic, bioinformatic, molecular, and immunological analyses	Develop/share improved and standardized protocols; organize workflows with core facilities
Extrapolation to human disease	Evaluating effects of life style on therapeutic outcomes	Consider gender, diet, and exposure to environmental factors in protocol development; consider effects of microbiota
	Physiological difference between mouse and human	"Humanize" aspects of mice; consider scaling law in PD/PK, lifespan, hemodynamics, etc.

case include: (1) a sufficient number of dedicated staff covering a broad range of expertise; (2) access to sophisticated instrumentation and technology for a full range of small animal imaging modalities, histological and molecular pathology, genomic technologies, pharmacological methods, model generation, and appropriate maintenance and quality control for a large "bank" of models; and (3) data management strategies. Examples of such organizations include the Center for Advanced Preclinical Research (CAPR; Center for Cancer Research, National Cancer Center and the Frederick National Laboratory for Cancer Research, https://ccr.cancer.gov/capr-home); Mouse Clinic for Cancer and Aging research (MCCA; Netherlands Cancer Institute and the European Research Institute for the Biology of Aging, http://www.mccanet.nl/); Center for Co-Clinical Trials (MD Anderson, http://www.cancermoonshots.org/platforms/center-for-co-clinical-trials/); and the Co-Clinical Project: Informing Clinical Trials Using Preclinical Mouse Models (Harvard Medical School). Similar efforts focused specifically on pancreatic ductal carcinoma include the Mouse Hospitals (Columbia Medical School, http://www.olivelab.org/mouse-hospital.html, and Cold Spring Harbor Laboratories).

Emerging and Future Prospects

This PRIMER focuses on the attributes and limitations of murine cancer models that currently best emulate our existing understanding of human cancers, an ever-expanding awareness of which is required to drive development of effective preclinical platforms. The high cost and low yield of efficacious therapies, despite clinical evaluation of countless potential therapeutics, motivate the use and development of preclinical PDX and GEM in the guidance of clinical research. Ultimately, collective employment of a variety of model systems will likely be required to successfully impact clinical outcomes.

Optimal mouse studies are sufficiently cumbersome so as to preclude the simultaneous evaluation of numerous drugs and unbiased libraries; high-throughput in vitro screening systems are essential precursors to in vivo evaluations. Despite their limitations, cancer cell lines have proven valuable in uncovering mechanisms of acquired drug resistance for in vitro drug screens (Torrance et al., 2001), and several technologies such as RNAi and CRISPR/Cas9 methods have enhanced their versatility (Corcoran et al., 2013; Shalem et al., 2014). However, cancer cell-line screens identify only drugs that target intrinsic cancer cell functions. Targeting tumor stroma or microenvironment/tumor cell interactions requires the use of in vitro systems that approximate the composition of cancers that preserve important cancer constituents, cell-cell interactions, and architectural features. To this end, several ex vivo platforms have been developed, including spheroids, organoids, microtumors (tumor tissue in synthetic matrix), and tissue slices (Burdett et al., 2010; Mendoza et al., 2010; Yamada and Cukierman, 2007). While optimization and validation of emerging ex vivo models in drug screening is ongoing, many may be incorporated into early phases of drug development, resulting in efficient triage and increased success in vivo.

In addition to ex vivo systems, non-murine whole organism drug screens have shown promise for early triage (Gao et al., 2014). Due to their relatively small size, low cost, and high fecundity, invertebrates such as flies (*Drosophila*) and nematodes (*C. elegans*) have shown promise. Furthermore, zebrafish (*Danio rerio*) are particularly well suited for high throughput screens because of rapid extra-uterine development, embryonic transparency, and recently developed pigment deficiency to facilitate imaging (Barriuso et al., 2015). Using automated high content and high throughput platforms, zebrafish can be used for chemical, genetic, and pathway-based screens (Lieschke and Currie,

2007). Notably, data generated from zebrafish models have been used in clinical trials. For example, the pyrimidine biosynthesis enzyme DHODH was identified in zebrafish screens as a novel melanoma drug target, and a clinical trial is underway in which patients are being treated with the DHODH inhibitor leflunomide (Hagedorn et al., 2014; White et al., 2011). Zebrafish have also been used as hosts for human and mouse xenografts to monitor invasiveness, angiogenesis, and drug responses in real time (Zhang et al., 2015). However, as with xenotransplantation of human cells into mice, inappropriate tumor-host interactions could limit the relevance and translational value of fish models.

Optimization of preclinical models that can impact clinical practice will require overcoming challenges in several arenas (Table 3). However, achieving this goal will undoubtedly require expansion and integration of organized efforts by many factions. The sophistication of such preclinical studies requires expertise in many disparate fields and necessitates involvement of scientists in the public sector, who often possess critical expertise and mechanisms not available in the private sector. However, communication and data-sharing among investigators and organizations, though essential for efficient optimization of effective preclinical standard operating procedures, are limited. A future priority will be to develop interactive web-based systems to house and mine experimental databases and SOPs for community sharing. Such organized initiatives will begin to meet the significant and immediate need to revolutionize the accuracy of preclinical assessment and to develop and utilize PDX- and GEM-based disease models in research to increase the number of effective treatments reaching clinical trials and thus, cancer patients.

In summary, we now have a wealth of model systems that show early promise in establishing robust preclinical assessment platforms for improving clinical success. Each system has specific and sometimes unique value, and all will undoubtedly play a significant role in varied aspects of future preclinical studies. At this junction, systematic comparisons in the prediction of human outcomes by distinct model systems has not been carried out and is needed in order to construct sound preclinical operating principles. The selection of models for a given study will undoubtedly depend on the required purpose. While 2D cell cultures are useful for identifying cancer cell-intrinsic vulnerabilities, 3D ex vivo methods incorporate assessment of multicellular interactions. Non-mammalian animals further offer reasonable throughput in complex biological systems, while PDX and GEM models provide the best representation of tumor microenvironments, physiological responses, and disease pathology. GEMs further allow for evaluation of immune system interventions and of responses unique to in situ developed disease. Ultimately, the complementary use of many of these models and continual efforts to improve their effectiveness will propel preclinical studies to a new era of cancer therapeutics development. This is a uniquely exciting era wherein preclinical models, rather than serving simply to confirm clinical outcomes, have the potential to routinely fuel optimized clinical success.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found with this file at http://dx.doi.org/10.1016/j.cell.2015.08.068.

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