Using Genetically Engineered Mouse Models of Cancer to Aid Drug Development: An Industry Perspective

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Abstract Recent developments in the generation and characterization of genetically engineered mouse models of human cancer have resulted in notable improvements in these models as platforms for preclinical target validation and experimental therapeutics. In this review, we enumerate the criteria used to assess the accuracy of various models with respect to human disease and provide some examples of their prognostic and therapeutic utility, focusing on models for cancers that affect the largest populations. Technological advancements that allow greater exploitation of genetically engineered mouse models, such as RNA interference *in vivo*, are described in the context of target and drug validation. Finally, this review discusses stratagems for, and obstacles to, the application of these models in the drug development process.

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As we approach 30 years since the dawn of recombinant DNA technology, the availability of the mouse genome sequence and of increasingly sophisticated tools for the manipulation thereof have resulted in the mouse being the model of choice to mimic the events that occur during human tumor initiation and progression (1). Currently, the standard anticancer drug development pipeline uses xenograft and/or orthotopic implantation of human tumor cell lines in immunocompromised mice as the primary *in vivo* models for investigating drug candidate efficacy and mechanism(s) of action (2). Although these models provide valuable information and somewhat greater predictability than cell/tissue cultures *in vitro*, their prognostic usefulness for human clinical trials has been limited. Genetically engineered mouse models (GEMM) of cancer are becoming useful systems for understanding the molecular and cellular determinants of tumorigenesis and for refining anticancer agents targeted to various facets of carcinogenesis *in vivo*. This review will focus on the criteria that define appropriate GEMMs as well as their availability for cancer types with the greatest effect on mortality and the preclinical applications thereof.

What Is a Good Mouse Model of Cancer? What Are the Tools for Making One?

Somatic alterations in oncogenes and/or tumor suppressor genes in normal tissues are thought to initiate human cancer, followed by a stepwise accumulation of molecular and cellular changes that ultimately result in a malignancy (3). During this progression, tumor cells interact with and co-opt various components of the surrounding microenvironment, including infiltrating immune cells, vascular and lymphatic networks, and the extracellular matrix (4). Modeling the interplay between progressively neoplastic cells and the surrounding tissue microenvironment is essential to accurately test therapeutics targeted at various players that support the tumor infrastructure (5). The correct reproduction of these events within a mouse requires genetic manipulations that faithfully recapitulate somatic events in adult humans in the appropriate population of adult mouse cells. In addition, the neoplastic progression resulting from these genetic perturbations in mice should phenocopy the human cancer at the histopathologic and molecular level, as well as respond similarly to known therapeutics for the disease. Finally, desirable logistical variables for models to serve as preclinical platforms include high disease penetrance and consistent, stepwise progression over a timeframe that is amenable to experimental therapeutics. Although many existing GEMMs do not meet all of these criteria, several of these models remain highly useful platforms for the mechanistic and therapeutic studies of pathway alterations within cancers, even if the evidenced disease does not completely reflect that in the human.

Techniques used for the facile introduction of alterations in the mouse genome range from germline (constitutive) transgenesis and gene knockouts to more common and sophisticated means of exerting temporal and spatial control of gene expression or attenuation (reviewed in refs. 6–8). Viruses offer additional means to mediate both spatial and temporal gene regulation; the two most commonly used are adenoviruses and retroviruses. Adenoviruses expressing Cre recombinase have been used to model lung and colon cancer (9–12). Another approach to deliver genes to somatic cells *in vivo* uses retroviral transduction via the TVA receptor for subgroup A avian leukosis virus (13, 14). The ability to introduce multiple potentially cooperating genes into a tissue of interest makes the RCAS/TVA system eminently attractive (15). Similarly, lentivirus-mediated transgenesis represents a powerful alternative to conventional technologies for making GEMMs (16). Methods to engender consistent viral infection and integration within embryos present

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significant challenges; however, the successful use of viruses as tools *in vivo* requires appropriate delivery to the target tissue or tumor of interest. Efficient viral infection has been shown in readily accessible adult organs, such as the skin, lung, and colon (9 – 12, 17); surgical techniques are currently emerging for virus delivery to additional internal organs, e.g., prostate (18) and pancreas,[1] thereby expanding the usefulness that these unique, titratable, and highly desirable systems offer.

Modeling High-Impact Cancers with GEMMs

Lung cancer. The majority of cancer deaths worldwide are due to lung cancer, which remains a relatively intractable disease in terms of both detection and treatment (19). Most lung cancers are associated with tobacco use, albeit the rate of incidence is rising in nonsmokers. Lung cancers are divided into two main classes based on clinical behavior and histologic criteria: non – small cell lung cancer (NSCLC), which afflicts ~80% of patients, and small cell lung cancer (SCLC), which accounts for ~20% of cases (20). NSCLC is further categorized into three main histologic subtypes: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. The differences between NSCLC and SCLC may be explained, in part, by the distinct patterns of chromosomal and/or gene alterations associated with each class. Genetic lesions associated with the initiation and promotion of NSCLC include mutations, amplifications, and/or overexpression of *K-RAS2* (~20-30%), *EGFR* (~20%), *c-MYC* (~8-20%), and *CCND1* (~40-50%), as well as alterations affecting the tumor-suppressor genes *P53* (~50%), *p16/INK4A* (~50-75%), and *RB* (~15-30%; ref. 21). The large variation in mutation incidences may reflect differences among histologic subtypes, gender, ethnicity, and/or differences between primary tumors and established cell lines. In contrast, some of the primary players involved in the etiology of SCLC include aberrations in the *RB* (~90%), *P53* (~80%), *p14/ARF* (~65%), and *PTEN* (~10-20%) tumor suppressors; *MYC* family DNA amplification (~25%); as well as mutations in the *c-MET* proto-oncogene (~12.5%; ref. 21).

Targeted activation of the *K-RAS* proto-oncogene (9, 12, 22, 23) and simultaneous inactivation of *RB* and *p53* (10) in the mouse lung have resulted in GEMMs that recapitulate many of the characteristics of NSCLC (subtype adenocarcinoma) and SCLC, respectively (Table 1; reviewed in ref. 24). Satisfyingly, the RNA expression profile of NSCLC in the $K\text{-}ras^{G12DLA/+}$ model shows a signature closely resembling that observed in human lung adenocarcinomas (25). These models have made significant strides toward modeling more aggressive lung disease and metastasis and will continue to improve as relevant mutations are analyzed in the appropriate combinations (26). Lung cancers show a high frequency of different histologic subtypes within the same tumor, leading to the hypothesis that these lesions arise from a common progenitor/stem cell – like compartment within the airway epithelium. The recent discovery of the bronchioalveolar stem cell and its role in the development of lung adenocarcinoma in the $K\text{-}ras^{LSLG12D/+}$ mouse model of NSCLC (27) could prove invaluable in further refining tumor classification and aiding our understanding of lung cancer etiology and biology. In summary, these more refined mouse models of lung cancer fulfill several of the criteria described for GEMMs. What remains to be elucidated in these models are the contributions of other mutations found in the human diseases, the identity of the cell type(s) of origin of SCLC, and the logistics of holding out large-scale RNA interference (RNAi) and preclinical trials examining experimental therapeutics of interest.

Breast cancer. Mammary cancer is the most common tumor in women and the second leading cause of female cancer deaths in the Western world (19). The disease progresses through distinct histologic stages associated with multiple genetic alterations, including *P53* loss (~50%), *PI3K* mutation (~30%), amplification and misexpression of *ERBB2/HER2* (~30%), *C-MYC* (~15%), and *CDKN2A* (~10%; ref. 28). Loss of one of the tumor suppressor-genes *BRCA1* and *BRCA2* is strongly linked to familial cases of breast cancer, which make up 3% to 8% of all diagnosed cases. Most breast cancers are classified as one of four major subtypes: ER (estrogen receptor)$^+$/PR (progesterone receptor)$^+$/Her2$^-$, ER$^+$/Her2$^+$, ER$^-$/Her2$^+$, and ER$^-$/PR$^-$/Her2$^-$; molecular profiling of tumors has also revealed additional subtypes (29). ER-positive tumors are responsive to therapies that selectively modulate estrogen, such as tamoxifen and raloxifene. Twenty-five percent to 30% of metastatic breast cancers that overexpress ErbB2/Her2 can be successfully treated with Trastuzumab. Significant proportions of patients have triple-negative breast cancers, however, and are generally unresponsive to standard therapies, underscoring a serious need for good models for this malignancy.

Several GEMMs for breast cancer have been developed, the majority involving overexpression of oncogenes via the long-terminal repeat of the mouse mammary tumor virus (reviewed in ref. 30). Some of the overexpressed genes in these models are *c-Myc*, *cyclin D1*, *Her2*, and *Wnt-1*, the latter indicating a role for this pathway in mammary tumorigenesis (31). In particular, tumors overexpressing Wnt-1 seem to show heterogenous ER status, making the model relevant to the study of both ER-positive and ER-negative malignancies (32, 33). Although most GEMMs for breast cancer develop ER-negative tumors, a recent study by Wijnhoven et al. (34) presents evidence that the dominant-negative $P53^{R270H}$ allele can predispose mice to ER-positive mammary tumors. Some of the challenges in making physiologically relevant GEMMs for mammary cancer are the shortage of endogenous promoters specific to the breast epithelium and the complications introduced by promoters that are hormone and pregnancy regulated. Recent reports by Hu et al. (35) and Welm et al. (36) describe a model that uses the isolation, manipulation, and transplantation of primary mouse epithelial cells; the latter study identified a role for the c-Met oncoprotein in mammary carcinogenesis and showed cooperation with c-Myc in the generation of heterogenous lesions that express early mammary progenitor cell markers (37). This system presents an interesting alternative to the abundance of conventional models, many of which exhibit notable differences in pathology and metastasis from the human disease. Extensive studies have gathered and compared the gene expression patterns in several GEMMs of breast cancer, resulting in a list of tumor signature genes, subclusters of which are specific to particular models or groups of models (38). Several technical hurdles, however,

[1] M. Singh et al., unpublished data.

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Table 1. Examples of models that currently emulate the GEMM criteria

Indication	Genotypic alterations	Type of allele(s)	Promoter/ context	Phenotype
Lung				
NSCLC	K-ras$^{G12D-LA2}$, K-ras^{LSLG12D}, or K-ras^{LSLG12V}	Conditional KIs or transgenic	Endogenous or β-actin; Adeno-Cre	Multistage disease progression from AAH to Ad to AdC
	K-ras^{LSLG12D} + p53^{LSLR270H} or p53^{LSLR172H} + p53^{Fl}	Conditional KIs or KO	Endogenous; Adeno-Cre	Disease progression is accelerated and more advanced, presenting with stromal desmoplasia and metastases to regional LNs and kidney Sinonasal AdC
SCLC	RB^{Fl} + p53^{Fl}	Conditional KOs	Endogenous; Adeno-Cre	Bronchiole hyperplasia; SCLC; metastases to bone, brain, adrenal gland, ovary, and liver
Breast	p53^{LSLR270H}	Conditional KI	Endogenous; WAP-Cre	Mice develop sarcomas and various carcinoma types, including adeno, solid, papillary, and adenosquamous DMBA treatment accelerates progression
	Wnt-1 ± p53-KO	Transgenic or conventional KO	MMTV	Multistage disease from ductal HYP to AdC, metastases to regional LNs and lung p53-KO accelerates disease progression Heterogenous ER status
	p53-KO	Conventional KO	Endogenous Syngeneic transplant	Occasional ductal HYP, moderate to poorly differentiated AdC AdC exhibited aneuploidy DMBA treatment accelerates progression
	c-MET ± c-MYC	Transgenic	Tet-inducible MMTV or MSCV-LTR Syngeneic transplant	c-MYC alone results in increased branching and HYP c-MET alone results in nonprogressing MIN Combination results in multistage disease from high-grade neoplasia to AdC

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Table 1. Examples of models that currently emulate the GEMM criteria (Cont'd)

Limitations	Possible future directions	References
Lesions do not recapitulate advanced human pulmonary AdC		(8, 11, 20, 21)
	Combine with other alterations associated with human NSCLC, AdC subtype Develop models that mirror other subtypes of human NSCLC	(24)
Yet to prove HYP lesions are precursors to SCLC Long timelines	Combine with other alterations associated with human SCLC Define stem cell	(9)
WAP-Cre expression is lactation dependent Sarcomas uncommon in human mammary cancer Long latency in absence of DMBA treatment	Combine with other alterations associated with human breast cancer Find improved mammary-specific promoters or methods to activate alleles	(33)
Transgenic overexpression of Wnt-1	Physiologic regulation of Wnt-1 Combine with p53-DN alleles Find improved mammary-specific promoters or methods to activate alleles	(30, 31)
Yet to prove HYP was a precursor to AdC Transplanted system Long latency in the absence of DMBA treatment	Combine with other alterations associated with human breast cancer	(33)
Transgenic overexpression Transplanted system	Combine with other alterations associated with human breast cancer Find improved mammary-specific promoters or methods to activate alleles Define stem cell	(34)

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Table 1. Examples of models that currently emulate the GEMM criteria (Cont'd)

Indication	Genotypic alterations	Type of allele(s)	Promoter/ context	Phenotype
Prostate				
	NKX3.1-KO + PTEN-KO + p27-KO	Conventional KOs	Endogenous	NKX3.1; PTEN compound mutants display multistage disease progression from HYP to LG-PIN to HG-PIN/early carcinoma, including metastases to LNs p27 loss enhances progression and is sensitive to gene dosage Androgen independence following androgen ablation
	$PTEN^{Fl}$	Conditional KO	Endogenous; probasin-Cre4	Multistage disease progression from HYP to PIN to invasive AdC, including metastases to draining LNs and lung Tumors regress following androgen ablation, but still proliferate in absence of androgen
	$PTEN^{Fl}$ + $p53^{Fl}$	Conditional KOs	Endogenous; probasin-Cre4	PTEN; p53 compound homozygous mutant mice display rapid onset, multistage disease progression from HG-PIN to lethal, invasive prostate cancer Loss of PTEN initiates prostate tumorigenesis, whereas p53 loss accelerates tumor progression Acute PTEN loss induces cellular senescence that is rescued by combined loss of p53
Colon				
	APC^{min}	ENU mutation	Endogenous	Ad and rare AdC in both the small and large intestines
	$APC^{\Delta716}$ + SMAD4-KO	KI or conventional KO	Endogenous	$APC^{\Delta716}$ lesions are larger and more advanced in combination with SMAD4 loss
	$APC^{Fl\text{-}580S}$	Conditional KI	Endogenous; Adeno-Cre	Colorectal Ad, with half demonstrating submucosal invasion (AdC)
Ovarian				
	p53-KO $\pm$ K-ras^{G12D}, c-MYC and/or AKT^{Myr}	KO or transgenic	Endogenous; β-actin or K5-TVA; Viral-LTR; syngeneic transplant	Undifferentiated epithelial neoplasms dependent on p53 loss and at least two cooperating oncogenes

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Table 1. Examples of models that currently emulate the GEMM criteria (Cont'd)

Limitations	Possible future directions	References
Long latency in the absence of DMBA treatment Incomplete penetrance Does not metastasize to bone Mice are susceptible to other neoplasms due to global KO, thereby influencing lifespan and limiting time for cooperating events to accumulate	Use Nkx3.1-Cre KI and combine with conditionally-regulated alterations associated with human prostate cancer Investigate mechanism of androgen-independent proliferation Define stem cell	(39, 40)
Does not metastasize to bone Incomplete metastatic penetrance	Combine with other conditionally regulated alterations associated with human prostate cancer Investigate mechanism of androgen-independent proliferation Address inconsistencies with other $PTEN^{Fl}$-driven prostate models	(41)
Lack of metastatic disease	Combine with other conditionally regulated alterations associated with human prostate cancer Address inconsistencies with other $PTEN^{Fl}$-driven prostate models Define stem cell	(37)
Predominantly small intestinal disease Does not progress to late-stage, metastatic disease Mice succumb to anemia due to high number of polyps	Combine with other alterations associated with human colon cancer	(51)
Predominantly small intestinal disease Does not progress to late-stage, metastatic disease	Combine with other alterations associated with human colon cancer	(55)
Consistency of delivery was not established Does not progress to late-stage, metastatic disease	Combine with other alterations associated with human colon cancer Define stem cell	(10)
Transplanted system Lower throughput due to surgical procedure Histology not well defined	Establish better-defined tumor histology/subtype Lower throughput due to surgical procedure	(56)

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Table 1. Examples of models that currently emulate the GEMM criteria (Cont'd)

Indication	Genotypic alterations	Type of allele(s)	Promoter/ context	Phenotype
	SV40 T and t antigens	Transgenic	MISIIR	Poorly differentiated epithelial carcinomas; differentiated parts resemble serous AdC Testicular tumors in male mice
	RB^{Fl} + p53^{Fl}	Conditional KOs	Endogenous; Adeno-Cre	Well-differentiated serous AdC Poorly differentiated CK8^{+} neoplasms Undifferentiated neoplasms Metastases to contralateral ovary, lung and liver
	K-ras^{LSLG12D}+/-PTEN^{Fl}	Conditional KI or KO	Endogenous; Adeno-Cre	Peritoneal endometriosis (specific to K-ras^{LSLG12D} alone) Endometriod ovarian AdC PTEN loss accelerates disease progression and results in lung metastases
Pancreas	K-ras^{LSLG12D}, p16/INK4A^{Fl}, p19/ARF^{Fl}	Conditional KI or KO	Endogenous; Pdx1-Cre	Multistage disease progression from PanIN to ductal AdC Progression to AdC requires p16/p19 loss Extensive metastases to intestinal tract, regional LNs, liver, spleen, adrenal gland, gall bladder, retroperitoneum, and diaphragm
	K-ras^{LSLG12D}, p53^{LSLR172H}	Conditional KIs	Endogenous; Pdx1-Cre or p48-Cre	Multistage disease progression from PanIN to ductal AdC, including desmoplastic response P53^{R172H} mutation conferred decreased latency and genomic instability Extensive metastases to lung, regional LNs, liver, adrenal gland, ascites, pleural surfaces, and diaphragm
Angiogenesis	SV40 T antigen	Transgenic	RIP	Multistage disease progression from HYP/DYS to AI to IC

NOTE: This table is intended to illustrate examples of models that fulfill some/all of the criteria that define improved GEMMs; it is not comprehensive.
Abbreviations: KI, knock-in; KO, knockout; HYP, hyperplasia; AAH, atypical adenomatous hyperplasia; MIN, mammary intraepithelial neoplasia; PIN, prostatic intraepithelial neoplasia; PanIN, pancreatic intraepithelial neoplasia; LG, low grade; HG, high grade; DYS, dysplasia; Ad, adenoma; AdC, adenocarcinoma; AI, angiogenic islet; IC, invasive carcinoma; ER, estrogen receptor; WAP, whey acidic protein; RIP, rat insulin promoter; MISIIR, Mullerian inhibitory substance type II receptor; LN, lymph node; DMBA, 7,12-dimethylbenz(*a*)anthracene; MMTV, mouse mammary tumor virus; LTR, long-terminal repeat.

have affected the ability to make meaningful comparisons between microarray-based gene expression profiles from mouse and human mammary tumors. Serial Analysis of Gene Expression analysis of p53-deficient transplant tumors in mice has identified 72 deregulated transcripts that are common between mammary tumors in mice and humans (35). Further comparative analyses are needed to validate existing GEMMs of breast cancer and guide the development of improved models.

Prostate cancer. Cancer of the prostate is the most common malignancy in men and the second leading cause of male cancer deaths (19). The molecular genetics of prostate carcinogenesis has been difficult to define and most data come from the analysis of chromosomal losses and candidate tumor-suppressor genes within these loci. These studies implicate the tumor suppressors *PTEN* (~10%), *RB* (~10%), *P27*, and *P16/INK4A* as well as perturbations in the Wnt, Hedgehog, and Notch developmental pathways in tumor initiation and progression (20, 21). Amplification of *MYC* and overexpression of several fibroblast growth factor family members, including FGF2, FGF7, and FGF10, are associated with progression in humans and mice (28). Acquisition of androgen independence and loss of *P53* are found in advanced carcinoma and metastasis; recently, p53-dependent cellular senescence has been shown to restrict tumorigenesis initiated by *PTEN* loss (39). *NKX3.1* is a homeodomain transcription factor that is down-regulated in up to 90% of both preinvasive and invasive prostate cancer cell (40). Prostate-specific inactivation of the mouse orthologue leads to prostatic intraepithelial neoplasia (41), indicating that *NKX3.1* may serve as a gatekeeper for prostate cancer analogous to the role of *APC* in colon carcinogenesis.

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Table 1. Examples of models that currently emulate the GEMM criteria (Cont'd)

Limitations	Possible future directions	References
Transgenic Driven by viral oncoproteins	Use the same promoter to drive Cre/LoxP–regulated conditional alleles associated with human ovarian cancer	(57)
Long latency Lower throughput due to surgical procedure	Develop similar models that mirror other types of human ovarian cancer Combine with other alterations associated with human ovarian cancer	(58)
Lower throughput due to surgical procedure	Develop similar models that mirror other types of human ovarian cancer Combine with other alterations associated with human ovarian cancer	(59)
Minimal desmoplastic response	Combine with other alterations associated with human pancreatic ductal cancer Address inconsistencies with other oncogenic K-ras–driven models of pancreatic ductal AdC	(62)
	Combine with other alterations associated with human pancreatic ductal cancer Address inconsistencies with other oncogenic K-ras-driven models of pancreatic ductal AdC Define stem cell	(63, 64)
Transgenic Driven by viral oncoprotein	Apply lessons learned from this model to more relevant disease indications	(66)

The identification of prostate-specific genes coupled with the generation of robust Cre-expressing mouse lines have enabled the prostate-restricted ablation of various tumor suppressors that cannot be fully investigated via conventional knockouts (due to embryonic lethality). As with breast cancer, modeling prostate carcinogenesis in the mouse is complicated by the morphologic differences between the human and mouse organs as well as the challenges involved in detecting and following metastasis, which is the ultimate cause of mortality in most humans. Historically, various transgenic models overexpressing SV40 large T antigen, or various oncogenes (e.g., Myc) and growth factors implicated in prostate cancer, have shown neoplastic initiation (28). Only a subset of these models, however, exhibit progression to invasive carcinogenesis and metastasis, albeit infrequently to the bone, the major site in humans. Recent compound analyses of prostate-specific conditional knockouts of the *NKX3.1*, *PTEN*, *P27*, and *P53* tumor suppressors show great promise for improved models of multistage disease and metastasis (39, 42, 43). Although the detailed characterization of these models is ongoing, these developments set the stage for further analyses of various players incriminated in these processes, and for the refinement of methods and markers for the detection of primary and metastatic lesions. Additionally, as in the mammary gland, the importance of the stroma in prostate tumor development (44) begs the need for the identification of means to manipulate this component *in vivo*.

Colon cancer. Gastrointestinal cancers, particularly those of the colon and rectum, are ranked third in the Western world in terms of annual new occurrences as well as the number of fatalities (19). Several of the common genetic lesions in colon cancer have been extensively mapped and studied (45). The most notable alterations include disruption of Wnt/β-catenin signaling via mutations in the *APC* gene found in ~90% of familial cancers and 30% to 50% of sporadic tumors, activation of K-RAS (~30%), and phosphatidylinositol 3-kinase (~20%), as well as loss of P53 function (~45%; refs. 20, 21). In addition, mutations in the SMAD family of proteins, particularly DPC4/SMAD4, are also found in ~40% of tumors and thought to result in altered transforming growth factor-β signaling (46). A small subset of hereditary tumors, named hereditary nonpolyposis colon cancers, relies on the loss of DNA replication fidelity as a key driver of the disease (47). Microsatellite instability is a hallmark of this subclass of tumors and is observed in ~15% of

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sporadic colon tumors, which also seem to harbor mutations in the transforming growth factor-β type II receptor (48). Diet and lifestyle also seem to play a significant role in the susceptibility to gastrointestinal neoplasia (49, 50).

The $APC^{Min/+}$ model was the first genetic model of intestinal tumorigenesis and continues to be one of the most used, particularly for chemopreventive studies (reviewed in refs. 51, 52). These and other *APC* mutant mice develop multiple intestinal adenomas as a result of similar mutations in the *APC* gene as found in patients that develop familial adenomatous polyposis (53). Key limitations of the mouse model(s), however, include the location of the polyps (predominantly restricted to the small intestine; ref. 54) and the rare occurrence of malignant progression to adenocarcinoma. Most *APC* mutant strains perish at ~14 to 16 weeks of age, afflicted with anemia from multiple polyp bleeds; colonic adenocarcinomas are rarely observed in these mice and metastases are absent. This abrogation of lifespan is thought to prohibit sufficient time for the accumulation of additional genetic lesions that cooperate in promoting later-stage disease. Indeed, the hypomorphic $APC^{1638N/+}$ mutant mice, which live beyond 1 year of age, develop fewer initial small intestinal polyps and subsequent malignant adenocarcinoma (55); selective *APC* attenuation in the large intestine results in adenoma and invasive adenocarcinoma of the colorectum (11). In contrast, the locations of intestinal lesions in mice with alterations in transforming growth factor-β signaling vary, in particular.

$SMAD3^{-/-}$ mice develop colonic adenocarcinoma and metastases, albeit in an *APC*-independent manner (56). Interestingly, $SMAD4^{+/-}$;$APC^{\Delta716/+}$ compound mutant mice present with more advanced intestinal lesions, supporting the hypothesis based on human genetic data that these two pathways cooperate during tumor progression (57). The high frequency of *APC* mutations in both premalignant lesions and sporadic colon cancer cases has implicated *APC* as a "gatekeeper" gene in gastrointestinal tumor initiation. Furthermore, genetic evidence suggests that the order of gene mutation is important in human colon carcinogenesis, i.e., mutations in *K-RAS* and *P53* cooperate in tumor progression but seem insufficient for tumor initiation (reviewed in ref. 45). Thus, accurate recapitulation of this progression in a GEMM will require careful spatially and temporally controlled introduction of additional genetic lesions onto an *APC* mutant background. Given the pleiotropic nature of transforming growth factor-β signaling, it will also be important to reproduce the exact mutations found in human tumors as opposed to complete deletion of the gene(s).

Ovarian cancer. Epithelial ovarian cancer is the deadliest malignancy of the female reproductive tract and ranks fourth in causing female cancer mortality (19). The current lack of means to accurately detect and diagnose ovarian neoplasia makes a strong case for the development of appropriate models for this disease. In the heritable form of ovarian cancer, women with mutations in *BRCA1* or *BRCA2* show an increased predisposition to ovarian tumors in addition to breast cancer; interestingly, ovarian cancers are also observed in patients with hereditary nonpolyposis colon cancer (see Colon Cancer section; ref. 20). Genetic alterations found in sporadic ovarian cancer include loss of *P53* (~55%) and *PTEN* (~5%) as well as mutations in *K-RAS* (~15%), *B-RAF* (~15%), and *β-catenin* (~10%; ref. 21). Overexpression of Her2/Neu, phosphatidylinositol 3-kinase, Akt, and c-Myc are also observed in tumors (28).

Various GEMMs for ovarian cancer generated over the last 2 years exhibit features of one or more of the predominant subtypes of the disease. Orsulic et al. (58) used the RCAS-TVA system to overexpress oncogenes in ovarian epithelial cells *ex vivo*, followed by implantation within the ovarian bursa. Both this model, and another in which female transgenic mice express the transforming region of SV40 under the control of the Mullerian inhibitory substance type II receptor gene promoter (59), develop poorly differentiated and undifferentiated tumors, with a percentage of serous adenocarcinomas, the major subtype observed in humans. A similar phenotype is observed in a virally induced model of simultaneous, Cre-mediated *P53*, and *RB* inactivation (60), and all three models show metastases consistent with human pathology. Recently, Dinulescu et al. (61) have used similar methodology to simultaneously activate and/or inactivate conditional alleles of $K\text{-}RAS^{G12D}$ and *PTEN*, respectively, within the ovarian surface epithelium. Approximately half of the $K\text{-}RAS^{LSLG12D/+}$ mice developed peritoneal endometriosis; the ovarian surface of all these mice and 62% of the $PTEN^{Fl/Fl}$ mice exhibited benign proliferative lesions with typical endometrioid glandular morphology. Importantly, all $K\text{-}RAS^{LSLG12D/+}$;$PTEN^{Fl/Fl}$ compound mice developed invasive endometrioid ovarian adenocarcinomas with many of the hallmarks associated with the corresponding human disease, including lung metastases (43%), thus supporting clinical data indicating a role for *PTEN* in the progression of ovarian endometriosis to endometrioid ovarian carcinoma (62). These last two models fulfill many of the criteria described for ideal GEMMs recapitulating sporadic carcinogenesis in adults and should greatly facilitate our understanding of the etiology and pathology of ovarian cancers.

Pancreatic cancer. Until recently, the dearth of histologically accurate models of pancreatic carcinogenesis represented a major stumbling block in the preclinical investigation of this lethal disease (63). Olive and Tuveson (8) present an overview of mouse models of pancreatic cancer and the GEMMs thereof in this issue; we summarize relevant GEMMs (64–66) in Table 1.

Tumor angiogenesis. One of the most successful molecular therapies to target cancer in the recent past has been the advent of antiangiogenic drugs (67). With the approval of bevacizumab (Avastin) and several other antiangiogenic agents currently in clinical trials, the need for improved models of tumor angiogenesis has become apparent. The most widely used and relatively predictive GEMM for tumor angiogenesis is the RIP1Tag2 model (68), a SV40 T-antigen-driven multistage pancreatic insulinoma model that has also served as a prototype for the design and execution of preclinical trials in the setting of multistep tumor progression (69). Similar principles have been applied to the testing of antiangiogenic drug candidates in an industry setting.[2] Studies conducted in the RIP1-Tag2 model set the precedent for the design of preclinical trials that yield valuable information on pharmacodynamics, pharmacokinetics, and the mechanism(s) of drug action in the endogenous tumor microenvironment.

[2] M. Singh et al., in preparation.

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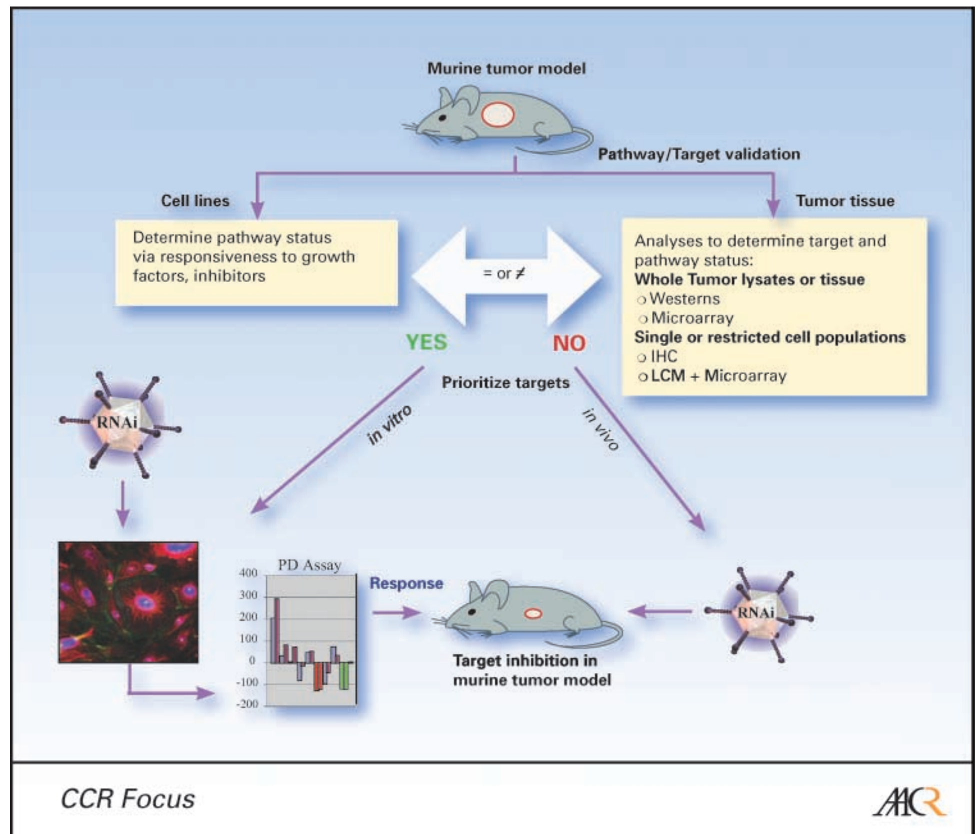

Fig. 1. Platforms for assessing and prioritizing target validation and mechanism of action *in vitro* and *in vivo*. Tumors from GEMMs can be used to generate cell lines and were analyzed directly via a number of diverse methodologies to examine target and pathway status. When a good correlation exists between the tumor cell lines and the endogenous tumors from which they are derived, these lines can be used *in vitro* to facilitate target validation, via RNAi and/or therapeutic response. In this manner, these lines can accelerate the target validation process by prioritizing which targets are pursued in the more cumbersome and labor-intensive efforts *in vivo*. PD, pharmacodynamic; IHC, immunohistochemistry; LCM, laser capture microscopy.

Using GEMMs to Inform and Facilitate Clinical Drug Development

Target and drug validation, mechanisms of action. The last decade has seen increased investigation of hitherto unexplored cancer pathways and the identification of an assortment of novel candidate target molecules for therapeutic intervention (3). GEMMs that accurately model the perturbation of such pathways can be used to fully explore the scope and consequences of these disruptions on the tumor cells and the surrounding milieu (5). These models also afford the unique ability to analyze the expression and role of a given cancer target/pathway at different stages of neoplastic progression.

Cell lines derived from tumors that arise in GEMMs present a unique opportunity to study the parallels and differences that arise in a tissue culture environment. Historically, it has not been possible to determine the relative contributions of tumor heterogeneity vis-a-vis the *in vitro* environment to the divergence of the genotypes and phenotypes in human tumor-derived cell lines. Because the GEMM tumor from which any line is derived is readily accessible, the genotype and phenotype of such cells can be constantly monitored for divergence from *in vivo* behavior. Also, tumor heterogeneity can potentially be recapitulated *in vitro* with the ability to derive multiple cell lines from a given model. Logistically, cell lines that preserve the pathway alterations found *in vivo* and consequent phenotypes *in vitro* can be used as first-pass screening tools to select and study candidate targets, pathways, and drugs for the more time- and labor-intensive *in vivo* experiments (Fig. 1). The use of RNAi, both *in vitro* and *in vivo*, is a significant advancement in the functional analysis of candidate genes involved in neoplasia (70). Short hairpin RNA (shRNA)–mediated gene knockdown can be used to interrogate the relevance of a target(s) or other pathway members of interest in tumor initiation, promotion, and survival, as well as interactions with other pathways. As depicted in Fig. 1, this validation can occur first in relevant tumor cell lines, if available, followed by analysis *in vivo*.

Theoretically, shRNA-mediated knockdown *in vivo* can be used to interrogate the role of a given gene in tumorigenesis (Fig. 2). Modeling multistage carcinogenesis to examine role(s) for genes of interest will require consistent, reproducible, and efficient shRNA delivery to the appropriate cell compartment in normal tissues, e.g., relatively rare progenitor cells in the hematopoietic lineage, the breast, or the brain, and in malignant tumors (71). Achieving these criteria in mammalian systems still presents significant technical challenges (16). Moreover, to mimic spontaneous tumor initiation in adult tissues, it will be important to regulate shRNA expression both spatially, via the appropriate promoter for a given tumor progenitor cell type, and temporally, such that RNAi induction occurs in adult tissues. Sophisticated viral-mediated technologies that couple tumor initiation with subsequent, inducible gene inactivation could potentially overcome mosaic shRNA expression that would likely result from the separation of these events (Fig. 2). Indeed, the key to making RNAi-mediated target/pathway validation in adult, solid tumors both tractable

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and informative will lie in achieving efficient, homogeneous knockdown throughout the tumor. In addition, it will be important to endow systems subjected to RNAi, whether endogenous or transplanted (xenografts and orthotopics), with a means of detecting targeted cells to facilitate the delivery and validation processes. The advantage of inducible RNAi in an endogenous system, such as a GEMM, is that it will allow the interrogation of a given gene in premalignant stages as well as in malignant tumors, i.e., knockdown of the gene(s) of interest can be timed to coincide with the neoplastic stage of interest; this is not possible in the context of transplanted human tumor cell lines. In summary, the ideal *in vivo* RNAi experiments would include facile shRNA delivery methods, means to identify the targeted normal or tumor cell type(s), and the ability to regulate the induction of gene knockdown and assay the consequences thereof.

The information gained from studies of the molecular components of cancer pathways in GEMMs is easily leveraged into the use of these models as systems for preclinical therapeutics with candidate drugs aimed at validated targets of interest. One can imagine a scenario wherein the neoplastic phenotype(s) resulting from RNAi aimed at a gene of interest can be compared with treatment with a drug candidate that targets the same gene and/or pathway, thereby directly testing the hypothetical mechanism of action of the therapeutic agent. Moreover, multiple shRNAs can be used to titrate target activity and gain knowledge of possible therapeutic windows (72). It is important to note the following caveats to this approach: (*a*) gene knockdown via RNAi is functionally distinct from target inhibition using a drug, and hence may not completely mirror drug action, and (*b*) tissue-specific RNAi does not model global, preclinical toxicity assessment (73). Nevertheless, target knockdown in an adult tumor tissue likely represents a more predictive model of a molecular therapeutic compared with germ line gene knockouts.

Although the predictive power and usefulness of GEMMs for oncology has become evident in the last decade, integrating these models into the drug discovery and development process remains a challenge faced by the translational research community. A schematic for this process is outlined in Fig. 3, with GEMMs being used to facilitate lead validation and mechanism of action studies. The throughput offered by xenograft and orthotopic models serves a valuable role in funneling the selection between various candidate drugs in the lead-optimization process before putting them through the higher bar of an endogenous microenvironment. A limitation of this systematic screening approach, however, is the possibility of missing candidates that would only be discovered by screening through a GEMM. Short-term pharmacodynamic assays in GEMMs can provide key insights into the specificity and mechanism of action of candidate molecules in the appropriate tumor milieu by identifying and distinguishing between various targeted cell populations, while simultaneously delineating tumor stage-specific effects of a particular therapeutic regimen (74). For example, experiments with small-molecule kinase inhibitors in the RIP1-Tag2 model have identified the contributions of at least two different cell types to the antiangiogenic effects of these agents (75).[2]

Facilitating preclinical and clinical assessments: pharmacodynamics, pharmacokinetics, and response. The development of

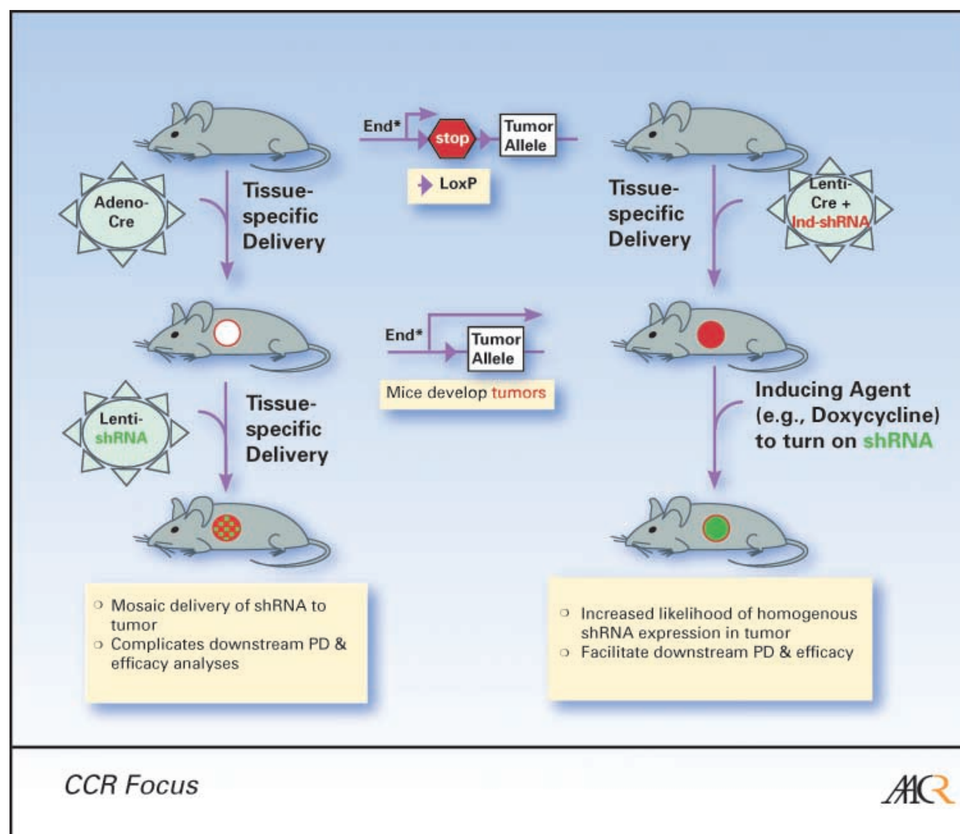

Fig. 2. Viral-mediated tumorigenesis and target validation. Adenoviruses have been successfully used to infect tissues of interest and deliver recombinases (e.g., Cre) to activate and/or inactivate LoxP-regulated alleles that mediate tumorigenesis. To support target validation efforts, the resulting tumor-bearing mice can subsequently be infected with lentiviruses expressing various shRNAs directed against targets of interest as shown on the left; however, efficient downstream PD and efficacy analyses will be hindered by mosaic shRNA delivery and subsequent expression throughout the tumor(s). One possible means to address this issue is through the development of novel viral vectors designed to coexpress the activating recombinase(s) and an inducible shRNA(s) (*right*). This would require the use of integrating viruses (e.g., lentivirus) such that the initial infected cell(s) would be "flagged" and the resulting tumor cell population would, in theory, all carry the same inducible shRNA events. This, in turn, would result in an increased likelihood of homogeneous shRNA expression throughout the tumor, thereby facilitating downstream validation efforts. End, endogenous; Adeno, adenovirus; Lenti, lentivirus; Ind, inducible.

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better predictive models and biomarkers for cancer therapeutics requires improved, mechanism-based pharmacokinetic/pharmacodynamic modeling and validation. Short-term pharmacodynamic assays examining experimental agents in mouse cancer models can be used to rapidly assess therapeutic effects and optimize those that provide predictive data on drug mechanism of action. As stated above, the setting of the endogenous model would be the ideal proving ground for testing mechanistic hypotheses and assays of drug action. If validated, such approaches can be used to guide and focus

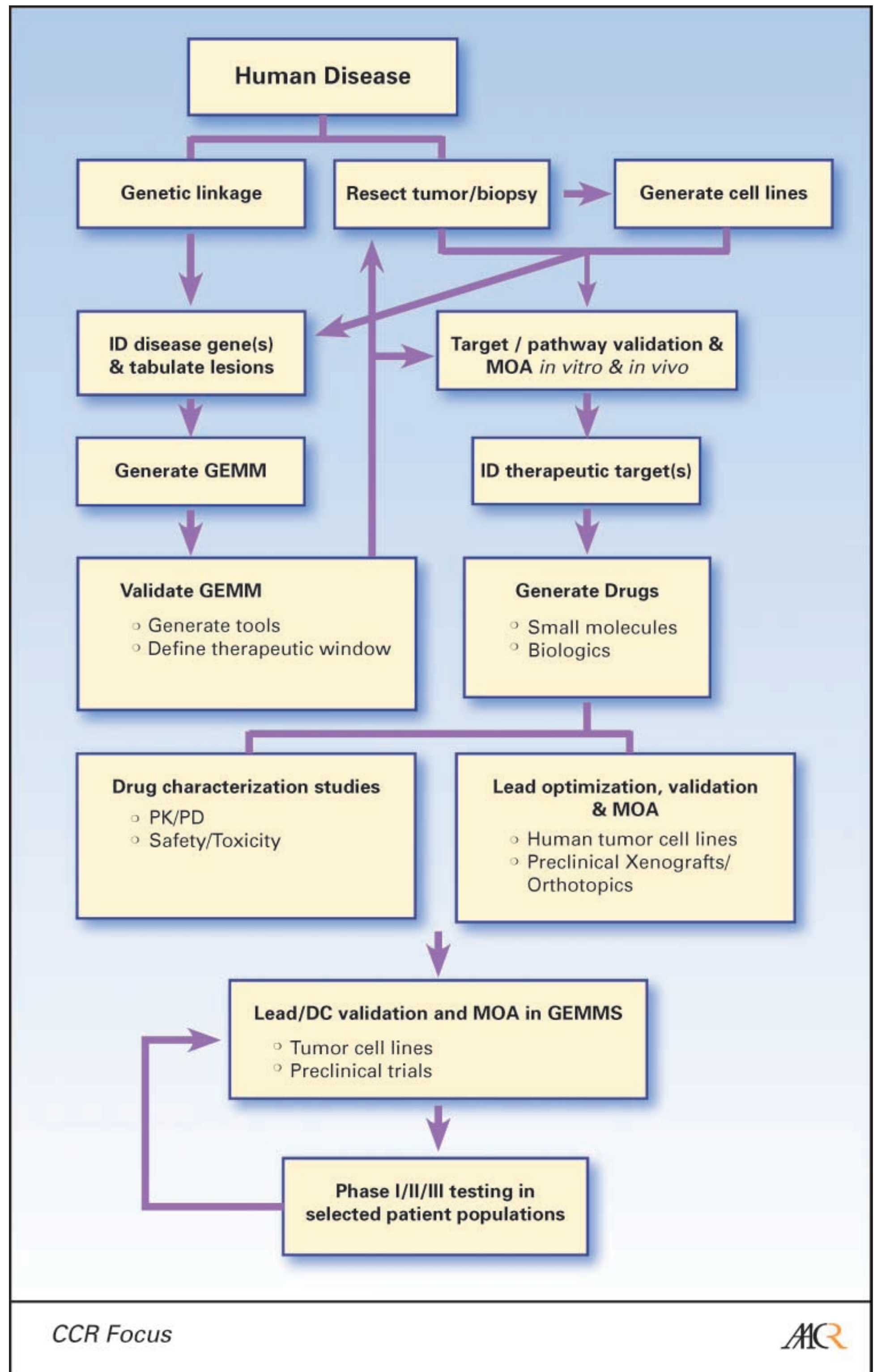

Fig. 3. Proposed roles for GEMMs in supporting the drug development process. The flow chart depicts the establishment of GEMMs carrying the mutation(s) identified in the human cancer they are designed to emulate and their application thereof. Following their generation and validation, these models can be used to further validate cancer targets/pathways as well as identify new targets. They ultimately provide a more solid foundation for aiding the downstream preclinical assessment of later-stage drugs (e.g., development candidates, *DC*) and could prove invaluable in defining both target and drug mechanism of action (*MOA*). Should they prove to be better prognostic indicators of clinical success than currently accepted modes of testing (e.g., xenografts), these models have the potential to significantly aid and inform the clinical process and ultimately alter current drug discovery paradigms used in the pursuit of novel therapeutics and appropriate drug combinations.

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the choice of drug, appropriate drug combinations, and dosing regimens in longer-term efficacy experiments that can be significantly expensive and resource-intensive. GEMMs that present with multiple, independent tumor-initiating events in the appropriate endogenous microenvironment offer several advantages for preclinical experiments, including the ability to investigate tumor heterogeneity and multistage disease within the same mouse. This ability to assay numerous lesions within the same mouse increases the statistical power and reduces the requirement for large numbers of mice in preclinical experiments.

GEMMs can vastly facilitate the discovery of tumor biomarkers that will serve as prognostic indicators as well as markers to monitor drug pharmacodynamics and efficacy. For example, the *K-ras*$^{LSLG12D/+}$ model of pancreatic cancer has been used to identify potential biomarkers of oncogene activation using a mass spectrometry–based serum proteomics approach (65). An alternative and equally interesting approach to biomarker identification is multianalyte profile testing, a technology consisting of a high-density immunoassay panel that is applied to serum/plasma followed by multivariate analysis of the profiles (76). Microarray profile data indicating that cathepsins are activated in two different GEMMs, lung adenocarcinomas in *K-ras*$^{LSLG12D/+}$ mice (77) and pancreatic insulinomas in the RIP1-Tag2 model (78), have inspired the use of activated cathepsin probes to optically label tumors that overexpress and activate these enzymes. Such approaches that couple molecular tumor signatures with powerful diagnostic tools can be used to sample tumor heterogeneity *in vivo* and guide tailored, patient- and tumor-specific therapeutic strategies in the clinic.

Sophisticated imaging modalities are needed to measure tumor growth within internal organs as opposed to traditional caliper measurements used in s.c. xenografts. Recent developments in small-animal imaging modalities have resulted in novel means to detect tumor markers and monitor preclinical trials in progress (79). Bioluminescent and fluorescent reporter mice are currently available for various tissues of interest, allowing the noninvasive imaging of both tumor growth as well as therapeutic response (reviewed in ref. 80). Such reporter mice can potentially provide significant savings in the time and cost of preclinical trials by providing faster readouts and reducing the number of animals needed. The disadvantages of such mice lie in the need to add one more genetic cross to already complex GEMM genotypes as well as the heterogeneity of reporter response and/or activation in different tissue and tumor types (81). The combination of multiple readouts using bioluminescence can increase the content of information gained, particularly in the case of pharmacodynamic reporters such as E2F1 for proliferation in ref. (82). The wider availability of small animal–adapted instruments for various imaging tools used within the clinic, such as micro–positron emission tomography, ultrasound (including Doppler ultrasound to monitor blood flow and tumor angiogenesis), magnetic resonance imaging, and *in vivo* X-ray micro–computed tomography, have great potential to inform the monitoring process in humans (83). Improved software programs are needed, however, for the quantitative analysis of small-animal imaging data in a high-throughput fashion, e.g., algorithms for tumor recognition and tumor burden calculation in X-ray images.

Challenges Ahead, What Needs to be Done

Xenograft models, the current workhorses of the oncology drug development process, are the most widely used platform for testing the efficacy and mechanism of action of drug candidates *in vivo* before their examination in humans in clinical trials (other mammals are used to inform dose tolerance and toxicity). These xenograft implants have several limitations as *in vivo* models of tumor progression, including the lack of an intact immune system, the inability to model premalignant neoplastic stages, and imperfect recapitulation of the interactions between tumor cells and the surrounding stroma (84). Although these models are clearly a step forward from *in vitro* experiments on cell lines that have been adapted to grow in culture, there remains room for improvement in the usefulness and predictive effect of *in vivo* models used for preclinical assessment of cancer therapeutics. Although GEMMs have this kind of usefulness and the potential to have significant predictive and economic effect on the process of anticancer drug discovery, these models are not extensively used in preclinical trials and have not gained wide acceptance in industry. Here, we list the barriers to the use of GEMMs in oncology drug development (Table 2).

The first significant obstacle to preclinical use of GEMMs in industry is the network of proprietary and commercial rights surrounding the use of these models. Currently, various patents surrounding the technologies required to create GEMMs, e.g., Oncomouse and Cre-lox, as well as the absolute cost of and time to procure the intellectual rights to mouse lines from various institutions, present major impediments to the introduction of these models in an industry context. Additionally, the intricacies of licensing compound genetic mice can easily involve legal negotiations with multiple institutions, the navigation of which adds even more time and resources to the acquisition of such rights. Although licensing fees are more of a barrier for smaller pharmaceutical companies, it is worth mentioning that such costs are merely the initial outlay for establishing GEMMs in a preclinical program; several considerations then come into play for the appropriate application of these models in drug development.

Second, the current generation of GEMMs on mixed and varied genetic strain backgrounds pose significant increases to the timelines associated with backcrossing these lines into a desirable, homogenous, inbred background before being able to apply them in the most meaningful and consistent manner in preclinical trials. Although accelerated backcrossing through speed congenics can help streamline these efforts, multiallele models can be subject to significant delays in application. Importantly, there is the potential for significant risk in changing the tumor phenotype and/or penetrance as lines are backcrossed into a common, inbred background. This lack of consistent strain backgrounds presents marked logistical and economic hurdles to moving a GEMM into the preclinical testing realm. Furthermore, in many cases, differences in barrier status at individual animal facilities, and the need to have mice free from specific pathogens (e.g., *Helicobacter* spp.), result in additional costs and time for embryo rederivation of GEMM lines. Institutional core facilities are equipped with the means to address these issues by taking on the generation and cryopreservation of single and multiallele embryonic stem cells and/or

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Table 2. Challenges facing GEMM implementation in drug development

Challenge	Specific barriers	Some possible actions
Intellectual property	Cost	Concerted voice on pricing criteria for mouse licenses
	Time to negotiate and acquire	
	Multiparty agreements	
	Bigger effect on smaller companies	
	Access to experimental agents from different companies	
Colony establishment	Genetic background differences	Employ one strain background (e.g., C57 Bl/6) universally
	Time to backcross	Develop lines upfront in ES cell backgrounds of choice to reduce backcrossing needs
	Implementation and associated cost of speed congenics	Create cryopreserved banks of ES cells and/or embryos of single and multiallele models*
	Pathogens in different colonies	Establish universal standards of acceptable pathogens (e.g., *Helicobacter*-free desirable)
	Rederivation: associated cost and time	
Preclinical trial implementation	Large-scale breeding space requirements	Use IVF for rapid colony expansion
	Resources for large-scale husbandry and genotyping	Bank large numbers of embryos for desirable lines
	Detailed time course analyses of tumor progression in desirable	Progression data documented[†] GEMMs
	Minimal data on SOC treatments in GEMMs for various cancer	Assemble database of SOC dosing regimens in relevant cancers GEMMs[†]
	Lack of ADME/PK data in immune-competent strains	Systematic survey of ADME/PK parameters for SOC and targeted therapeutics in commonly used strain(s)[†]
	Antibody therapeutics that do not cross react to mouse target(s)	Screen for species cross reactivity early in development and/or engineer "humanized" mice
	Noninvasive tumor imaging capabilities for trial enrollment and therapeutic response	Increase accessibility to instrumentation facilities
	Lack of appropriate reagents to measure targeted therapeutic responses (e.g., reliable phosphospecific antibodies)	Commercial efforts to generate sufficient quantities and access to improved and novel antibodies/markers
Data acquisition and analysis	Lack of means for high-throughput biomedical imaging	Technology development
	Lack of means for high-throughput microscopy	Improved software programs
		Establish standards for quantitative histologic variables

Abbreviations: ES, embryonic stem cell; SOC, standard of care; IVF, *in vivo* fertilization; ADME/PK, absorption, distribution, metabolism, and excretion/pharmacokinetics.

*Expand upon existing Mouse Models of Human Cancer Consortium efforts and establish in institutional core facilities; likely requires dedicated funding.

[†]Make available in public domain (e.g., Mouse Models of Human Cancer Consortium database).

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embryos from GEMMs in commonly used strain backgrounds. The Mouse Models of Human Cancer Consortium is carrying out an admirable job of collating a resource for information on GEMMs as well as providing some strains to the community; we strongly encourage the expansion of these efforts. Although such an enterprise may require specifically allocated funding, the savings in time and effort afforded by such a bank are worth the consideration of the scientific community.

Third, once a model is established as genetically and phenotypically homogenous, it must be thoroughly characterized and validated internally before being applied to preclinical trials. Initial published characterizations of models often lack the association of disease stages with relevant therapeutic windows, i.e., a GEMM usually has to be reexamined with the goal of identifying the appropriate intervals and end point(s) for simulating human clinical trials.

Last, preclinical experiments clearly require large numbers of mice to be readily and consistently available. The need for rapid expansion of mice can be partially addressed by *in vitro* fertilization, which, in turn, necessitates additional resources and access to the appropriate technologies. Taken together, the major barriers to bringing GEMMs into an industry setting are

as follows: (*a*) a common strain background for GEMMs, (*b*) the presence of complicating pathogens in various colonies, (*c*) a lack of model validation with an eye to preclinical trials, and (*d*) the framework for large-scale production colonies. Addressing these challenges within the scientific community would go a long way toward promoting a more rapid and broader implementation of these models in the drug discovery process.

Within the pharmaceutical world, the infrastructure for conducting large-scale preclinical experiments with GEMMs is still in evolution (85). One hurdle to a wider audience for GEMMs in the industry setting is a dearth of preclinical data using current therapeutic standards. There is a strong need for the validation of these models using conventional cytostatic and cytotoxic drugs, i.e., current standards of care, thus testing whether the responses observed in a given GEMM reflect those observed in humans when undergoing the same treatment(s). The examination of novel drugs, either alone or in relevant combinations, in GEMMs with naïve disease is highly informative for discerning and understanding drug activity. In the clinic, however, new agents are typically first tested as either monotherapies or in combination with the current standards of care against refractory or resistant disease. Thus, for these models to mirror the clinical process, the ideal GEMM would be one that would respond similarly to humans when treated with the current standards of care, and, in certain cases, develop resistant or refractory disease that subsequently enables the testing of appropriate combinations with investigational therapies. Furthermore, minimal data exist as to the metabolism of various experimental agents in the immunocompetent strains used to generate GEMMs. Thorough studies are warranted to assess whether notable differences exist between drug absorption, distribution, metabolism, and excretion, and pharmacokinetic variables in the immunocompromised mice typically used to grow xenografts and test cancer therapeutics versus different genetic strains of immunocompetent GEMMs. The authors strongly encourage the support and expansion of the Mouse Models of Human Cancer Consortium enterprise to include a database of preclinical trials and baseline data with conventional drugs and current standards of care in GEMMs.

An importunate consideration of using the mouse as a model for drug action is whether the target/pathway(s) is conserved at the molecular level. Whereas many small-molecule drugs (e.g., kinase inhibitors) cross-react with the mouse target, biologics present a unique challenge to assessing experimental therapeutics in GEMMs in that antibody specificity often does not cross the species barrier. A classic example is that of bevacizumab (Avastin), an antibody that binds and blocks human vascular endothelial growth factor-A signaling and is one of the most successful molecularly targeted anticancer drugs in recent years. Yet, it has not been possible to study bevacizumab in GEMMs as the drug does not bind mouse vascular endothelial growth factor-A. Cases like this have inspired some industry groups to consider the use of appropriate surrogate antibodies or the creation of humanized mouse knock-ins of the gene of interest. Knock-ins of human orthologues in mouse models raise, however, the specter of additional mouse lines and colonies to manage as well as the possibility that the human protein will not interact with the full spectrum of partners required for activity in the mouse. Ideally, the design of the anticancer drug development process would allow for the early testing and selection for biologics and small molecules that exhibit species cross-reactivity. Altogether, establishing the infrastructure to adequately support and conduct preclinical trials that fully evaluate both drug pharmacology and efficacy in GEMMs is no small feat and is very much a work in progress.

Finally, improved reagents and technological advancements are needed to expedite high-throughput data acquisition and downstream analysis of histopathologic data from preclinical testing in GEMMs. This is the most rate-limiting step once preclinical trials have initiated; the need for reliable diagnostic markers and assays is of course not confined to GEMMs, albeit the models face additional challenges in reagent availability and development, e.g., antibodies to phospho-epitopes that cross-react to mouse proteins. Although some automated microscopy systems and robotics for image acquisition are emerging onto the market, significant improvements are still needed. In addition, methods for the quantitation of histologic data show significant variation in the literature, illustrating a need for consensus standards for the numerical representation of histopathologic assays.

Although the intellectual property rights surrounding GEMMs hinder the application of these models in industry, the patents held by the biotechnology and pharmaceutical corporations limit access to novel antitumor agents for preclinical trials. Such regulations prevent the use of the full gamut of clinical competitors and present marked barriers in the design of meaningful preclinical studies in which therapeutic agents can be compared directly with competing clinical entities. Studies of this nature would have a significant effect upon informing the choices of clinicians and aiding the identification of appropriate patient populations for clinical trials. The recent Merck versus Integra ruling addressing this issue potentially opens the way to diminishing these impediments (86). Interestingly, because the Food and Drug Administration does not mandate preclinical efficacy in GEMMs as part of investigational new drug applications, the pharmaceutical industry does not always see the case for using these models, particularly given the time and cost of therapeutic experiments in these systems. Although some progress in addressing all of these issues has been made, the wider acceptance and application of GEMMs in the cancer drug discovery process requires a fundamental cultural change in the interactions between academia and industry regarding the use and application of GEMMs (87). Such a paradigmatic switch, perhaps facilitated by organizations like the Mouse Models of Human Cancer Consortium, could, in turn, accelerate the translation of valuable research in these models into improved therapies for cancer patients.

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