Illuminating Cancer Systems with Genetically Engineered Mouse Models and Coupled Luciferase Reporters In Vivo

Brandon Kocher and David Piwnica-Worms

ABSTRACT

Bioluminescent imaging (BLI) is a powerful noninvasive tool that has dramatically accelerated the in vivo interrogation of cancer systems and longitudinal analysis of mouse models of cancer over the past decade. Various luciferase enzymes have been genetically engineered into mouse models (GEMM) of cancer, which permit investigation of cellular and molecular events associated with oncogenic transcription, posttranslational processing, protein–protein interactions, transformation, and oncogene addiction in live cells and animals. Luciferase-coupled GEMMs ultimately serve as a noninvasive, repetitive, longitudinal, and physiologic means by which cancer systems and therapeutic responses can be investigated accurately within the autochthonous context of a living animal.

Significance: Luciferase-dependent bioluminescence imaging coupled with genetically engineered mouse models of cancer permit interrogation of tumor biology and therapeutic response within the proper physiological context of the whole animal in vivo. Cancer Discov; 3(6); 616–29. © 2013 AACR.

INTRODUCTION

Transitioning into the Proper Context

Genomic lesions within incipient cancer cells in collaboration with alterations in the microenvironment contribute to neoplastic progression (1–3). Tumor cells can modulate the surrounding microenvironment to promote the progression of cancer through intrinsic oncogenic pathways. Furthermore, key genetic lesions have a profound impact on cancer cell migration, invasion, and regulation of the immune system through tumor-extrinsic manipulation of the microenvironment (4, 5). The importance of the host microenvironment in neoplastic progression, independent of tumor manipulation, is also underscored by studies showing that fibroblasts, among many other stromal and immune cell types, stimulate growth of preneoplastic and neoplastic cells and promote drug resistance (6–8). Given these observations, understanding the complex interactions between genomic lesions and tumor microenvironment in mouse models is crucial to uncovering new anticancer therapies.

Thus, implementation of molecular imaging within basic research and preclinical mouse models of cancer has become an essential tool for interrogating these hallmarks of cancer and monitoring tumor progression within the proper physiologic context.

Currently, the most commonly used types of mouse models of cancer can be grouped into primary tumor cells, tumor cell lines, and their associated tumor engraftments, or genetically engineered mouse models (GEMM) of spontaneous cancer. Xenograft models entail subcutaneous or orthotopic transplantation of human cell lines or primary tumors into an immunodeficient mouse, whereas mouse allografts similarly use orthotopic or subcutaneous host implantation. Although traditional cell line xenografts and mouse allografts have yielded limited clinical correlations (9–11), the robust ability of human “xenopatient” models (12) and newly adapted “human-in-mouse” (HIM) cancer models for accurately modeling patient disease and predicting patient response have been encouraging (11–13). However, due to the inherent nature of xenograft and HIM models, immune-compromised mice are required, and thus the contribution of the immune system and the autochthonous tumor stroma cannot be fully interrogated. In addition, these tumors are implanted as a population of late-stage tumorigenic cells and do not accurately recapitulate all steps of tumorigenesis. In contrast, GEMMs permit investigation of the proper tumor microenvironment, model tumor development from the initial genetic alteration in situ to subsequent neoplastic progression to metastasis, and enable tissue-relevant drug pharmacodynamics (13). Constitutive or conditional GEMMs of cancer (transgenic, knockout, or knockin) as

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Luciferase Reporter Mouse Models of Cancer

**KEY CONCEPTS**

- Deciphering the complex molecular interactions between cancer somatic lesions and host microenvironment is crucial to understanding cancer cell proliferation, migration, invasion, and immune evasion and may guide new anticancer therapies.
- Of equal importance as digitized genomics, the next essential challenge is to functionize the cancer genome and correctly capture these molecular mechanisms in their proper biologic context within cancer systems.
- Molecular imaging with genetically encoded imaging reporters, especially bioluminescence, provides a dynamic and noninvasive analytical platform to resolve the cooperative genetic and environmental elements of cancer systems at various temporal and spatial scales.
- Fluorescent markers expressed in live cells and mouse models of cancer have provided powerful tools to monitor cancer-associated genetic circuits, signaling pathways, cell lineage, microenvironment interactions, and drug-targeted protein function in real time in vivo.

well as chimeric or nongermline GEMMs have proven to be of significant interest for cancer biology research as well as accurate predictive models of human cancers for preclinical drug development (14–16).

**Molecular Imaging with Genetically Encoded Reporters**

Regardless of the mouse model, molecular imaging techniques (nuclear, fluorescence, and bioluminescence) at both macroscopic and microscopic scales make it possible to explore the consequences of the interactions between tumor cells and microenvironment during tumor progression in vivo, in real time. This expanding set of molecular probes, detection technologies, and imaging strategies, collectively termed molecular imaging, now provides researchers and clinicians alike with new opportunities to visualize gene expression, biochemical reactions, signal transduction, protein–protein interactions, regulatory pathways, cell trafficking, and drug action noninvasively and repetitively in their normal physiologic context within living organisms in vivo (17–21). In particular, integration of genetically encoded imaging reporters into live cells and, more importantly, whole animal mouse models of cancer has provided powerful tools to monitor cancer-associated molecular, biochemical, and cellular pathways in vivo (22). Traditional means of interrogating these oncogene-associated biologic processes and characterizing new anticancer therapeutics have relied on invasive techniques that are often laborious and provide only a static window of analysis. Microscopic fluorescence imaging with GFP provided pioneering studies of biologic activities and cellular processes at high resolution (23). Concurrently, molecular probes, contrast agents, exploitation of fundamental tissue characteristics, and development of multi-spectral fluorescent and bioluminescent (luciferase) proteins and highly sensitive instrumentation have revolutionized noninvasive and longitudinal imaging of cancer biology at the whole organism level.

These various imaging modalities and strategies acquire macroscopic information in vivo through 2 basic strategies: injected agents or genetically encoded reporters. Injected agents have contributed significantly to preclinical cancer research and also have great potential for translation but require significant optimization and characterization depending on the experimental model, biologic target, background noise, instrumentation, and route of administration, and, for human use, are affected by similar regulatory hurdles as therapeutic agents (21, 22). An inherent constraint to the development of conventional injectable agents is that the details of synthesizing, labeling, and validating a new and different ligand for every new receptor or protein of interest impose long cycle times on development. However, genetically encoded reporters offer more modular tools for preclinical research, which, once cloned into appropriate vectors and biologically confirmed, can be quickly applied to a broad array of applications with minimal modification (22, 24). While genetically encoded imaging reporters are under development for use in humans, the potential for immunogenicity and transduction inefficiencies raises unique challenges (25). However, genetically encoded imaging reporters represent a technically and biologically robust means of monitoring the dynamics of tumor biology with relatively high temporal resolution and various levels of spatial resolution when coupled with GEMMs.

Imaging of biologic processes using genetically encoded reporters relies on the ability of the reporter gene to produce a measurable signal that can be detected and quantified by extrinsic instrumentation. Reporter expression and thus signal output is controlled by a regulatory element such as a constitutive or conditional DNA-promoter system, or subsequent peptide fusion that regulates posttranslational modulation of the reporter. Most commonly used genetically encoded imaging reporters produce a signal through optical imaging strategies, but magnetic resonance imaging (MRI) and radiopharmaceutical [positron emission tomography/single-photon emission computed tomography (PET/SPECT)] approaches have been explored. Optical imaging of genetically encoded reporters can provide image contrast through (i) reporter-mediated enzymatic activation of an optically silent substrate (e.g., light-producing luciferase-based oxidation of α-luciferin in the presence of Mg²⁺, ATP, and O₂; refs. 22, 26), (ii) photoexcitation signal production (e.g., fluorescent proteins; ref. 23), or (iii) reporter-mediated enzymatic release/trapping of optically tuned leaving groups [e.g., β-glucuronidase–mediated hydrolysis of glucuronide groups coupled to near-infrared (NIR) imaging dyes (27)]. Nuclear imaging of genetically encoded reporters can use (i) enzyme-mediated modification of a labeled substrate causing intracellular accumulation or proximal cell association (e.g., HSV1-TK–mediated phosphorylation of radiolabeled nucleosides for PET imaging; refs. 28, 29) or (ii) direct import of a labeled tracer (e.g., sodium iodide transporters/radiodines for PET/SPECT; refs. 22, 30). An early innovation for MRI was use of a galactopyranose-blocking group coupled to a gadolinium-based relaxivity agent that rendered the MRI contrast agent sensitive to expression of the reporter gene β-galactosidase (31).
Genetically encoded reporters with optical outputs, specifically fluorescence or bioluminescence, are most commonly used for cancer research in mouse models due to overall modest cost, sensitivity, and lack of the technical restrictions and required regulatory barriers often encountered with other approaches. Whole animal fluorescence imaging \textit{in vivo} suffers from low signal-to-noise as a result of background autofluorescence, modeling-dependent photon quantification, photobleaching, low tissue penetration, and low resolution (26). In addition, fluorescent proteins are known to generate reactive oxygen species (ROS) that can induce significant cellular stress under selected conditions (23). However, computed image analysis along with laser-induced fluorescence has increased the sensitivity of non-invasive fluorescence imaging \textit{in vivo} (32). Also, compared with other genetically encoded reporters, fluorescent proteins are independent of substrate delivery and pharmacokinetics and are amenable to high-resolution microscopic analysis, and several new far red-shifted fluorescent proteins have been developed that enhance the penetration of photons \textit{in vivo} (21).

Bioluminescence imaging (BLI) has emerged as an invaluable optical imaging tool and has become widely adapted to molecular imaging of cancer models \textit{in vivo}. The major advantages of luciferase reporter systems \textit{in vivo} include essentially zero background signal, high signal-to-noise imaging, relative ease of signal acquisition, modest cost, user-friendly instrumentation, and direct measure of live cell mass (ATP-dependent activity). Moreover, luciferase enzymes have a shorter half-life (~3–5 hours for native North American \textit{Photinus pyralis} firefly luciferase and \textit{Renilla} luciferase versus 12–26 hours for native GFP variants) and are rapidly folded and functional posttranslationally, thereby providing a more robust readout of kinetic processes such as transcriptional activation, protein degradation, reversible protein–protein interactions, and other rapid biologic processes (22, 33, 34). Also, red-emitting firefly and click beetle luciferases with relatively higher photon outputs have advanced luciferase imaging beyond the original long wavelength luciferase variants, providing further advantages over \textit{Renilla/Gaussia} luciferases and related mutants that emit at blue or blue-green wavelengths, which, while perhaps useful, remain subservient for imaging \textit{in vivo}. However, luciferase enzymes in general are dependent on substrate pharmacokinetics, and, furthermore, the \textit{Renilla/Gaussia} substrate (coelenterazine) is transported by P-glycoprotein and auto-luminesces due to auto-oxidation from serum albumin (35, 36), which can confound analysis \textit{in vivo}. In addition, due to overall low photon output, luciferase reporters traditionally have been limited to macroscopic imaging analysis. However, recent advances in low-light microscopy technologies have permitted the interrogation of live cells and live bioluminescent tissues \textit{ex vivo} at high magnifications (37–39), a notable advance that extends the capacity of BLI.

Similar to studies in cultured cells, genetically encoded bioluminescent reporters in mice offer the ability to noninvasively monitor transcriptional regulation and posttranscriptional and posttranslational events, as well as transformation and neoplastic progression. When coupled with an oncogenic protein or signaling pathway of interest, these properties of BLI allow various features of cancer to be interrogated, such as chemoresistance, inflammation, angiogenesis, DNA maintenance, apoptosis, therapeutic response, and oncogene addiction. In addition, BLI reporter mice can simultaneously and directly assess tumor burden through constitutive or conditional expression of luciferase. With the sensitivity of BLI reporter mice, one can also noninvasively survey and monitor small, nonpalpable tumors as well as metastases in a relatively fast and efficient manner. While a considerable amount of early effort was directed toward fluorescent GEMMs of cancer, only more recently have mouse models been advanced with the versatility of BLI. Thus, the development and usage of BLI in GEMMs of cancer is the focus of this review, and the reader is referred elsewhere for overviews of molecular imaging in non-GEMM models (19–22, 40).

More specifically, this review highlights GEMMs of cancer reported in the last half-decade that use genomically encoded bioluminescence reporters for investigating tumor biology and associated signaling pathways inherent to the cancer system or pathway of interest, summarizes notable models, and suggests future directions for BLI-coupled GEMMs of cancer. In addition to the models highlighted in detail later, an extensive referenced list of cancer-related luciferase-coupled GEMMs according to mode of luciferase regulation (Table 1) and cancer type (Table 2) are included for the general reader.

### REGULATION OF LUCIFERASE IN GEMMS OF CANCER

#### Transcriptional

Transcriptionally regulated luciferase enzymes provide a robust tool to monitor tissue-specific tumor burden or interrogate biologic processes in tumors \textit{in vivo}. Transcriptional systems are simple in design and consist of a composite or endogenous promoter sequence upstream of luciferase and introduced into the mouse genome either through transgenic or targeted knockin approaches. Conventionally, genetic regulatory elements derived from cytomegalovirus (CMV) or simian vacuolating virus 40 (SV40) provide robust protein or reporter expression in the cell or tissue harboring the construct. However, at the whole animal level, to track spontaneous tumor progression, researchers have used promoters or \textit{cis} acting regulatory regions from endogenous or viral genes activated specifically in neoplastic cells. For example, prostate growth and development is largely governed by androgen signaling, thus offering an avenue to specifically image prostate cells in physiologic or pathologic states. With this in mind, a plethora of transgenic luciferase mice have been developed using composite promoters from human kalikrein 2, probasin, prostate-specific antigen (PSA), and various forms of concatenated minimal androgen response elements (Table 1). Other transgenic models also use endogenous androgen-responsive promoters derived from rat probasin and human PSA (41, 42). Baseline signal with varying intensities is confined to the prostate with minimal promoter activity outside of the prostate for most models, allowing noninvasive prostate-specific BLI. Prostate bioluminescent signal from these promoters correlates with normal prostate development and decreases upon castration or androgen ablation. However, in only 1 transgenic model (Tg\textit{PSA>Luc}) did the reporter mouse show an increase in prostate bioluminescence when crossed to a previously characterized transgenic adenocarcinoma mouse prostate (TRAMP) model that expresses oncogenic SV40 small and large T antigen in the prostate via a minimal rat probasin promoter (42). Two other similar prostate transcriptional...
## Table 1. Cancer-associated processes observable in genetically encoded luciferase reporter mice

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<th>Cancer biology target</th>
<th>Genetic strategy</th>
<th>Ref.</th>
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<td>B-cell-specific imaging</td>
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<td></td>
<td>Various</td>
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<td></td>
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<td>Angiogenesis</td>
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<td>Various</td>
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<td></td>
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Abbreviations: DEN, diethylnitrosamine; KI, knockin mouse; RE, response elements multiple; Tg, transgenic mouse; TK, thymidine kinase; →, promoter-driving gene expression. Gene loci are separated by a semicolon (;).
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<td>Systemic response to antiandrogen therapy</td>
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<td>SV40 ER-induced pancreatic cancer</td>
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<td>Tet-off</td>
<td>HPV16 E7-induced cervical cancer</td>
<td>Tg(Krt15 promoter→rtTA; tet-o-Luc-E7)</td>
<td>(102)</td>
</tr>
<tr>
<td>Spontaneous</td>
<td>Transcriptional</td>
<td>Ubiquitous TAg-induced tumorigenesis</td>
<td>Tg(CAG promoter→flox-STOP-pA→flox-TAg-Luc)</td>
<td>(99)</td>
</tr>
<tr>
<td></td>
<td>Cre recombination</td>
<td>Whole animal stochastic Notch1 LOH</td>
<td>KI(Notch1 promoter→Notch1-Cre; Notch1-IRES-flox-Rosa26 promoter→flox-STF-flox-CBR)</td>
<td>(71)</td>
</tr>
</tbody>
</table>

Abbreviations: DEN, diethylnitrosamine; KI, knockin mouse; RE, response elements multiple; Tg, transgenic mouse; TK, thymidine kinase; -, promoter-driving gene expression. Gene loci are separated by a semicolon (;).
Luciferase reporter models failed to show a consistent increase in prostate signal when crossed to TRAMP models, despite histologically confirmed tumor progression (43, 44). It was suggested that the inability of these reporter models to show a tumorogenic increase in prostate bioluminescence, as seen in Tg(PSA->Luc; rPB-Tag) mice, was due to the androgen independence of these aggressive, neuroendocrine carcinomas that are characteristically observed on an FVB background (45). However, this observation is unsatisfying given the fact that the tumors were truly androgen-independent, the relatively high prostate-specific signal (>10^6 photons) would be, by default, representative of tumor mass only and would have been expected to increase along with the tumor, which was not observed despite tumor progression (43, 44). These discrepancies point out the potential pitfalls of using transgenic strategies with regulated promoters to noninvasively monitor tumor biology and development \textit{in vivo} with decreased signal variability compared with that often observed between transgenic founder lines. Although technically more difficult, knockin strategies maintain the entire promoter regulatory region adjacent to the luciferase gene, and thus, in principle, provide a more accurate measure of gene transcription within the native context of the genome. This is exemplified in a p21\textsuperscript{Fluc}/p21\textsuperscript{WAF1/CIP} knockin luciferase model (p21\textsuperscript{Fluc}) in which firefly luciferase was genetically introduced into the endogenous p21 locus downstream of the native promoter (Fig. 1A; ref. 37). p21 is a critical regulator of cell-cycle progression, is a direct transcriptional target of p53 among many other signaling pathways, and is frequently altered in human cancers (46). As expected, p53-dependent activation of the p21 promoter in response to external beam irradiation could be noninvasively and repetitively monitored over time in \textit{p21\textsuperscript{Fluc}} mice (37) using surgically implanted micro-osmotic pumps that constantly delivered d-luciferin substrate (ref. 47; Fig. 1B). Previous attempts to noninvasively monitor p21 \textit{levels} \textit{in vivo} used transgenic transcriptional luciferase or lacZ reporter mice that were regulated by short fragments of the p21 promoter (<5 kb) and produced robust signal only when strains harbored multiple copies of the reporter (up to 23; refs. 48, 49). In the knockin reporter strain, baseline bioluminescence levels 3 logs higher than the luciferase transgenic strain allowed Tinkum and colleagues (37) to identify specific organs that contained high levels of p21 independent of p53 status. In addition, selected organs showed dramatic regional differences in p21-luciferase activity that were identified using
high-resolution bioluminescence microscopy to localize to live suborgan structures and specific cell populations with high-level expression of p21 (Fig. 1C), providing new insight into future lines of investigation.

Similar to organ-specific imaging in the aforementioned prostate transgenic models, subcellular whole-animal imaging of tumor burden can be accomplished using transcriptional reporter mice as well. Scotto and colleagues (50) introduced a mCherry-Luciferase fusion into the endogenous Cd19 locus. This enabled noninvasive longitudinal imaging and microscopic analysis ex vivo of the B-cell lineage under normal and pathologic conditions when crossed to a λ-MYC transgenic mouse model of spontaneous B-cell lymphoma (50). Knockin strategies seem to offer more refined imaging of whole organs or distinct cell lineages in terms of sensitivity and specificity when it comes to analysis of GEMMs of cancer at the macroscopic scale.

However, a caveat is that knockin strategies can potentially disrupt expression of the endogenous locus by usurping its promoter function or preventing expression of the targeted locus, thereby disrupting the process under investigation. In this regard, several groups have adapted viral internal ribosome entry sites (IRES) to couple translation of luciferase enzymes to the transcriptional activation of an upstream gene in a bicistronic fashion while maintaining expression of the targeted gene. One group used this strategy by knocking in an IRES-EGFP-luciferase fusion downstream of the endogenous VEGF receptor 3 (VEGFR3) stop codon (Vegfr3EGFP luc; Fig. 2A; ref. 51).

Figure 2. Imaging inflammation and tumor-associated lymphangiogenesis. A, schematic representation of the Vegfr3 EGFP luc reporter knocked in downstream of the endogenous Vegfr3 locus, which uses an IRES for monitoring VEGFR3 expression. B, DMBA/TPA-induced skin papillomas in Vegfr3EGFP luc/+ reporter mice displayed localized lymphangiogenesis as indicated by the black arrows. C, whole body imaging of tumor-activated lymphangiogenesis over time in a B16-V5 melanoma xenograft model at distant lymph nodes (red arrows) before metastasis of the primary tumor xenograft in female Vegfr3EGFP luc/+ reporter mice. Images from Martinez-Corral and colleagues (51) modified and reprinted with permission from Proceedings of the National Academy of Sciences of the United States of America.
VEGFR3 is a potent regulator of angiogenesis, lymphangiogenesis, and metastasis and is emerging as an alternative target in combination with other VEGF anticancer therapeutics (52–54). The authors used this reporter mouse to quantify the association between inflammation and lymphangiogenesis during wound healing and in response to a constant hypoxia-sensitivity inflammation model. Microscopic analysis of the coupled EGFP reporter allowed the authors to show that Vegf26bp reporter mice maintained background photonic levels and thus, luciferase expression is a direct readout of this transfectional activation of essential UPR response genes (57, 58).

Importantly, the sensitivity and longitudinal capability of luciferase imaging permitted identification of tumor-activated lymphangiogenesis at distant lymph nodes that preceded tumor metastasis (Fig. 2C).

Posttranscriptional

The luciferase enzyme can also be coupled to posttranscriptional mechanisms that monitor mRNA modifications associated with oncogenic signaling. This can be accomplished by coupling or fusing a luciferase enzyme to a coding sequence such that modification or interaction of the mRNA or protein results in modulation of luciferase signal. This strategy was applied to investigate the effect of the tumor microenvironment on tumor unfolded protein response (UPR) and endoplasmic reticulum stress in live animals by visualizing alternative splicing (55). The heterogeneous tumor microenvironment imposes endoplasmic reticulum stress upon the tumor through hypoxia, acidic pH, and low nutrients (56). These constraints, along with deregulated translation and proteotoxicity, place evolutionary pressure on developing cancer cells, which can respond by augmenting several key steps of the UPR pathway for survival. Thus, UPR is an emerging target for anticancer therapies (56). Upon loss of endoplasmic reticulum homeostasis, activation of inositol-requiring enzyme 1α (IRE1α), 1 of 3 UPR pathways, results in unconventional splicing of a 26 bp intron from X-box–binding protein 1 (XBP-1), thereby incorporating an extended open reading frame that increases protein stability and augments XBP-1-mediated transcriptional activation of essential UPR response genes (57, 58). Spiotto and colleagues (55) created a transgenic mouse that harbors a CMV–XBP1-Luc transgene, which is regulated in a manner similar to that of the endogenous XBP-1, and thus, luciferase expression is a direct readout of this splicing event (55). Under normal physiologic conditions, the reporter mouse maintained background photonic levels but appropriately displayed tumor-specific signal once crossed to breast cancer models, such as Tg(MMTV-TAg) and Tg(MMTV-Her2), which localized with endoplasmic reticulum stress markers upon microscopic analysis of tumors. Importantly, the authors observed no correlation between tumor size and bioluminescent signal suggesting XBP-1–Luciferase was a measure of the tumor-intrinsic endoplasmic reticulum stress and not overall tumor load. Tumors arising within the same mouse possessed variable signal intensities, which portrayed the heterogeneous nature of tumor metabolism, as further indicated by differential glucose uptake and hypoxia, and the contribution of the unique tumor microenvironments within the same mouse. These observations serve as an example of the sensitivity of BLI compared with other optical imaging modalities that were used in a similar transgenic Tg(XBP1-GFP) mouse, which had very low signal that at the time only allowed endpoint analysis of XBP-1 activation in a few extracted organs (59).

Posttranslational

Cancer-associated posttranslational modifications and the subsequent effects on protein processing and protein–protein interactions are amenable to luciferase reporter mice and BLI in general. Previous designs and biologically affirmed reporters have transitioned from cell culture to provide the framework for whole animal preclinical evaluation of anticancer drugs in the proper physiologic context. Interrogation of inhibitors of the hypoxia-inducible factor-1α (HIF-1α) have been aided by the development and application of various transgenic mouse models that fused the oxygen-dependent degradation domain of HIF-1α to firefly luciferase [Tg(ODD-Luc) and Tg(Hypoxic RE>ODD-Luc); refs. 60–62]. Under normoxic conditions, endogenous HIF-1α protein is retained at low levels due to hydroxylation, polyubiquitination, and subsequent proteasomal degradation, and correspondingly, luciferase background signal levels are low in these luciferase reporter mice during normoxia (63). During hypoxia or hydroxylase inhibition, HIF-1α is stabilized and, appropriately, bioluminescence intensity of these reporter mice increases as a result, thereby indirectly monitoring HIF-1α–dependent responses to acute or chronic hypoxia. When crossed to spontaneous Tg(MMTV-neu) or carcinogen-susceptible RasH2 cancer models, increased bioluminescence was detected in hypoxic tumors, highlighting the ability to monitor both tumor growth and tumor hypoxia noninvasively with this reporter mouse (60, 62). In addition, inclusion of cis acting hypoxia response elements before a minimal CMV promoter in the Tg(HRE>ODD-Luc) mice inherently provided interrogation of the transcriptional phase of the HIF-1α–positive feedback loop, which attempts to recapitulate HIF-1α–dependent transcriptional activation of its own mRNA (62).

The analysis of druggable oncogenic protein–protein interactions can also be interrogated noninvasively using luciferase reporter mice. This notion is exemplified in a proof-of-principle mouse model using the Gal4-VP16 “two hybrid” interaction system in which nuclear interaction of the DNA-binding domain of the yeast transcription factor Gal4 with the transactivation domain from the herpes simplex virus VP16 protein can functionally lead to the transcriptional activation of a Gal4-responsive reporter gene (64). If each component is individually fused to a set of proteins known to interact, visualization of their proximity and interaction can be indirectly assessed through Gal4-responsive reporter activation. Pichler and colleagues (64) generated a transgenic mouse harboring a luciferase reporter regulated by Gal4 response elements, which could indirectly monitor protein–protein interactions by hydrodynamic somatic gene transfer of constructs expressing Gal4 fused to p53 and VP16 fused to the SV40 large T antigen. Using this reporter mouse, abrogation of p53–Tag interaction due to loss of p53 was readily observed in mice upon short hairpin RNA (shRNA)-mediated
knockdown of Trp53 in vivo. Adapting this modular system to other models of protein interactions could aid in the preclinical evaluation of modulators of oncogenic protein–protein interactions longitudinally in whole animals (65).

Conditional Transformation

Exquisite genetic techniques have enabled researchers to initiate and follow transformation, progression, invasion, metastasis, therapeutic response, and oncogene dependence in spontaneous or conditional GEMMs of cancer. Luciferase reporter mice can be genetically coupled to these molecular and biologic events, thus permitting longitudinal and noninvasive imaging of a relatively small cohort of mice that can provide statistically meaningful results, as each animal serves as its own control. In one example, inactivation of the retinoblastoma (RB) tumor suppressor pathway in response to platelet-derived growth factor (PDGF)–induced oligodendrogliomas was indirectly monitored through activation of a E2F1 promoter driving luciferase in an engineered transgenic reporter mouse (66, 67). Direct monitoring of genetic deletion of tumor suppressors or activation of oncogenes is also possible through the use of several floxed firefly luciferase transgenic mice in which Cre-mediated excision of an upstream floxed-stop cassette allows downstream luciferase expression. Previously, Lyons and colleagues (68) generated a transgenic strain in which the β-actin promoter-driven luciferase expression is regulated by removal of a floxed GFP-polyA. Crossing the reporter strain with a floxed GFP-nomas that could be simultaneously monitored using BLI followed by adeno-Cre inhalation induced lung adenocarcinomas was indirectly monitored through activation of a transgenic strain in which the β-actin promoter-driven luciferase expression is regulated by removal of a floxed GFP-polyA. Crossing the reporter strain with a floxed Kras2i12 followed by adeno-Cre inhalation induced lung adenocarcinomas that could be simultaneously monitored using BLI for over 100 days. Using the same reporter mouse crossed to a conditional prostate-specific Cre-expressing strain, Tg(PB-Cre4);Ptenfl/fl, another group was able to monitor for over 400 days spontaneous prostate adenocarcinoma initiation, progression, response to castration, and subsequent development of castration-resistant prostate cancers (CRPC) reliably in a small cohort of animals (69). The emergence of CRPC was not observed and potentially may never be observed through coupled-reporter gene imaging in the androgen-dependent transcriptional prostate carcinoma reporter models discussed earlier. This highlights the use of Cre/loxP approaches, which can mark tumor cell lineage before biologic-specific functions, thereby improving upon the complexities of transcriptional reporters, such as the prostate cancer reporter mice discussed earlier. The authors observed that the nonrecombined β-actin promoter-driven reporter was leaky and read-through could be observed in muscle tissue using reverse transcriptase PCR of tissue mRNA. This was potentially due to the strength of this promoter in regions of high actin expression and/or from the variability associated with the locus or extent of genomic integration of the reporter. Also, the authors observed pronounced luciferase signal at intrapelvic sites of repeated luciferin injection, which could be remedied through tail vein injection of the luciferase substrate. Svensson and colleagues (70) also used a conditional Rosa26-Luc knockin reporter mouse to monitor prostate carcinoma progression on the less aggressive C57BL/6 background, using the same strategy as Liao and colleagues [Tg(PB-Cre4);Ptenfl/fl, ref. 69]. Despite the now well-characterized differences in prostate development due to the genetic background of the mouse used (e.g., C57BL/6 vs. aggressive FVB/N), Svensson and colleagues noted a dramatic reduction in luciferase signal variability over time compared with Liao and colleagues. These differences potentially stem from the Rosa26 locus and/or the fact that the Rosa26-Luc allele was strategically backcrossed onto an albino C57BL/6 background, thereby greatly reducing signal attenuation due to coat color. In addition, analysis of Tg(PB-Cre4);Ptenfl/fl prostate tumors indicated the BLI was a more accurate readout of prostate tumor burden because ex vivo analysis revealed massive fluid retention in the anterior prostate that could be misinterpreted as tumor mass when analyzed via MRI.

Cre/loxP-luciferase reporter mice have also been used for following stochastic neoplastic genetic lesions and marking distinct cell lineages when coupled to other fluorescent or LacZ reporter strains. Liu and colleagues (71) implemented this strategy to identify organ susceptibility and monitor subsequent tumor progression in a sophisticated whole animal Notch1 loss-of-heterozygosity (LOH) model crossed to a floxed stop cassette-Rosa26-click beetle red luciferase (Rosa26→CBR) knockin reporter mouse (Fig. 3). The authors generated a mouse harboring a knockin nonfunctional Notch1-Cre fusion on one allele along with a conditional Notch1 knockout cassette on the second allele (Notch1flf), which was further crossed with either the conditional Rosa26→CBR mouse or other conditional Rosa26→LacZ and Rosa26→EYFP reporter mouse strains (Fig. 3A). Following the first round of embryonic Notch1 expression, any second endogenous ligand-dependent activation of Notch signaling in Notch1-Cre;Notch1flf mice results in cleavage and subsequent nuclear translocation of the Notch-intracellular-domain (NCID)-Cre fusion, which in turn results in excision of the remaining floxed Notch1 allele throughout all active Notch1-signaling cells (Fig. 3A). Thus, stochastic LOH and the subsequent development of highly vascularized tumors could be indirectly monitored at the macroscopic (Rosa26→CBR) and at the cell lineage level (Rosa26→LacZ/EYFP) by Cre-mediated excision of the coengineered lox-stop-lox cassette inserted upstream of the Rosa26 locus on each allele in these reporter mice (Fig. 3B and C). The Rosa26→CBR mouse has since been crossed with the Rosa26→LacZ mouse to create a conditional Rosa26→CBR/LacZ dual-modality reporter mouse that has been extensively backcrossed onto the albino C57BL/6 background (Pwnica-Worms and colleagues; unpublished data). This dual reporter mouse has the potential to provide a robust and powerful readout of Cre-activation and carcinogenesis through the high, red-emitting photonic output of CBR and the microscopic use of LacZ staining.

Tet-Regulated Systems

Conditional cancer mouse models using genetically coupled luciferase reporters can also use tetracycline (tet) (or the more stable analog, doxycycline)–regulated expression systems or tamoxifen-inducible systems for spatial and temporal induction and reversion of oncogene biology in mice. However, tet-systems are more commonly used due to toxicities associated with tamoxifen and the Cre-estrogen receptor fusion observed in tamoxifen-inducible
Luciferase Reporter Mouse Models of Cancer

In the tet-on system, expression is dependent on binding of a reverse tet-regulated transactivator (rtTA) engineered upstream of the coding sequence of interest. The tet-off system uses a different tet-regulated transactivator protein (tTA), which cannot bind tet-o in the presence of tet, effectively silencing expression only when tet is present. Regardless of the system, expression of the tet transactivator (and hence the gene of interest) can be regulated through promoters specific to a tissue or cell type of interest. These systems have been instrumental in determining the extent of oncogene dependence in spontaneous mouse tumor models within the proper context in vivo. In addition, conditional repression of an oncogene mimics therapeutic inhibition in a targeted molecular pathway, and coupled luciferase reporters allow longitudinal imaging confirmation of this “therapeutic inhibition.” In one example, Du and colleagues generated a conditional tet-o-PyMT-IRES-luciferase reporter mouse, Tg(tet-o-PyMT-IRES-Luc), to investigate the cell-specific effect of oncogene induction on the development and progression of pancreatic cancer using 2 previously reported conditional Tet-system mice Tg(Rip7-rtTA and Pdx1-tTA). Within 1 day of doxycycline removal, noninvasive BLI imaging allowed confirmation of subsequent oncogene withdrawal in Tg(Rip7-rtTA; tet-o-PyMT-IRES-Luc) mice. Interestingly, this had no effect on the established hyperplastic β-cell islets, indicating oncogene independence, which was also conversely confirmed using the Pdx1-tTA mice. In addition, 10% of Tg(Pdx1-tTA; tet-o-PyMT-IRES-Luc) mice developed aggressive acinar cell carcinomas as a result of activation of the Pdx1 promoter in early pancreatic progenitor cells. Regression of these tumors when deprived of PyMT, confirmed as soon as 1 day after doxycycline addition by BLI, indicated a requirement or dependence on PyMT for sustained tumor maintenance. Another group generated a tet-off luciferase reporter mouse to delineate the requirement of MYCN in medulloblastoma. One of 2 lines crossed to generate this bigenic mouse contained the cerebellum-specific glutamate transporter1 (Glt1) promoter driving expression of rtTA and the other a tet-o-driven bidirectional MYCN and firefly luciferase expression cassette. Using a characterized amount of photon flux as a measure of tumor burden, the authors repressed MYCN expression in a subset of mice

**Figure 3.** Imaging whole animal Notch1 LOH and surveying subsequent tumor development. **A,** schematic representation of Notch1-Cre;Notch fl; Rosa26-CBR cells before Notch1 ligand activation. **B,** upon activation, cleavage of NOTCH1 (S2 and S3) permits translocation of the NCID-Cre fusion into the nucleus where it excises both the remaining wild-type Notch1 fl allele, and the floxed stop cassette preceding CBR. Through these series of Cre-mediated excisions, lineage tracking of Notch1 LOH can be longitudinally imaged via the genetically coupled floxed Rosa26-CBR reporter. **C,** whole animal imaging of the development and progression of Notch1 LOH-induced angiosarcomas of the liver as imaged in Notch1 fl or Notch1 fl mice crossed to Notch1-Cre;Rosa26-CBR reporter mice. Images from Liu and colleagues (71) modified and reprinted with permission from Journal of Clinical Investigation.
by feeding them doxycycline-containing chow. Within 1 week, they observed a dramatic drop in bioluminescence and tumor regression, confirming the requirement for sustained MYCN expression in these tumors and suggesting its potential for targeted therapy in medulloblastomas. Thus, bioluminescent reporter mice coupled to integrated conditional tet-regulated systems are invaluable tools to quickly and efficiently monitor oncogene expression in manageable cohorts of mice and validate potential therapeutic targets, which guide and inform more invasive and laborious secondary tumor analyses.

Lessons Learned and Future Considerations
As with all experiments, meticulous planning and a dose of foresight are paramount to the success of engineering a genetically encoded luciferase mouse. There are several considerations and nuances that can dramatically reduce time and labor and expand upon the potential of a reporter mouse in terms of signal sensitivity, biologic accuracy, and overall use. As the genetic background of the mouse strain can dramatically affect the biology of the cancer, for example, as seen in the differential sensitivity of C57BL/6 and FVB strains to prostate cancer models as well as other models (79), so will genetic background inherently modulate the signal strength and interpretation of coupled luciferase reporters. When possible, an albino mouse strain should be used to minimize signal attenuation. This will allow for enhanced sensitivity for gross analysis of luciferase expression, and will strongly benefit low luciferase-expressing tissues in scenarios such as low endogenous expression, metastasis, or tumor regression. Commercial albino C57BL/6 embryonic stem cells are now available that have high germline rates and are technically amenable to genomic manipulation for targeted or transgenic reporter approaches, thereby minimizing onerous backcrossing. When specifically attempting to monitor live tumors, a relevant genetically encoded reporter should be specific to live cell mass alone and will likely be most accurate when using Cre/loxP-based or knockin strategies as discussed previously. Although technically much simpler, transgenic transcriptional reporters can be overridden by tumor evolution or genomic loci effects as seen in the androgen-sensitivity GEMMs of prostate cancer discussed earlier. Low-light microscopy, which is capable of imaging live luminescent tissues, is becoming accessible to the general researcher and is approaching the high levels of magnification and resolution necessary for subcellular inspection. Nonetheless, analysis of live tissues and organs synergizes with the whole-animal imaging capabilities of luciferase reporter mice and also can be conducted using luciferase antibodies or secondary coupled reporters, such as fluorescent proteins as used to observe lymphatic vessels in Vegfr3EGFP mice (51).

CONCLUSIONS
Genetically encoded luciferase reporter mice have made a profound impact on imaging tumorigenesis, cancer progression, response to therapies, and the contributions of the tumor microenvironment when crossed with GEMMs of cancer. Compared with other imaging modalities, bioluminescence provides an efficient, relatively low-cost, noninvasive, and longitudinal means to investigate genetic alterations in the autochthonous tumor environment and their ultimate effect on tumor biology. Combining the advantages of genetically encoded luciferase reporters with the development of new and clinically accurate GEMMs of cancer paints a bright horizon for our understanding of molecular cancer biology and the development of novel and durable anticancer therapies.

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Authors’ Contributions
Conception and design: B. Kocher, D. Piwnica-Worms
Development of methodology: B. Kocher
Analysis and interpretation of data (e.g., statistical analysis, bioinformatics, computational analysis): B. Kocher, D. Piwnica-Worms
Writing, review, and/or revision of the manuscript: B. Kocher, D. Piwnica-Worms
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): B. Kocher, D. Piwnica-Worms
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Brandon Kocher and David Piwnica-Worms


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