Review

Cancer Research

Genetically Engineered Mouse Models: Closing the Gap between Preclinical Data and Trial Outcomes

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Abstract

The high failure rate of late-stage human clinical trials, particularly in oncology, predicates the need for improved translation of preclinical data from mouse tumor models into clinical predictions. Genetically engineered mouse models (GEMM) may fulfill this need, because they mimic spontaneous and autochthonous disease progression. Using oncogenic *Kras*-driven GEMMs of lung and pancreatic adenocarcinoma, we recently showed that these models can closely phenocopy human therapeutic responses to standard-of-care treatment regimens. Here we review the successful preclinical application of such GEMMs, as well as the potential for discovering predictive biomarkers and gaining mechanistic insights into clinical outcomes and drug resistance in human cancers. *Cancer Res; 72(11); 2695–700. ©2012 AACR.*

Introduction

The conventional oncology-drug–development process involves the extensive use of preclinical mouse models to gauge the efficacy of new molecular entities (NME) *in vivo*. Historically, these models have predominantly, if not exclusively, relied on xenograft systems using human cancer cell lines and tumor explants in immune-deficient mice (1, 2). However, the results obtained with these surrogate model systems over the past several years have inadequately predicted human clinical outcomes, particularly in the case of targeted therapeutics (3, 4). Some notable exceptions to this are tumors that are clearly dependent on cell-autonomous pathway perturbations, such as mutations in epidermal growth factor receptor [EGFR (5)] or Type I, ligand-independent mutations in the Hedgehog signaling pathway (6). Nonetheless, these systems imperfectly model the autochthonous tumor microenvironment as well as the dynamic and evolutionary interplay between different cell types within tumors (i.e., paracrine interactions). Therefore, it is critical to identify more predictive and prognostic preclinical models of human malignancies to enable the development of new and effective cancer therapies.

Possible reasons for the disparate therapeutic responses observed in human tumors and mouse xenografts include the fundamental differences between mice and humans (such as drug metabolism, pharmacokinetics, toxicities, and combination tolerability), the particular attributes selected for during xenograft propagation, the incomplete nature of the xenograft tumor microenvironment, or some combination thereof. Some of these issues may be resolved through the use of genetically engineered mouse models (GEMM) of cancer, which more faithfully recapitulate many aspects of their corresponding human disease (7, 8). Preclinical models that more accurately phenocopy human disease (or subsets thereof) would enable investigators to better test drug efficacy and model mechanisms of action and therapeutic resistance. In addition, they could aid in the identification of optimal dosing regimens, both alone and in combination with other agents. The latter is particularly relevant because targeted NMEs are routinely combined with standard-of-care chemotherapeutics and, in recent clinical trials, with other NMEs.

Until recently, despite preclinical studies described in the literature, it was unclear whether GEMMs could actually predict human responses in the clinic better than xenografts, or how they might be made to do so. Part of the problem is that most of those preclinical studies did not adequately model the human clinical scenario with fidelity. Specifically, (i) the mutations they used did not always phenocopy the appropriate physiological context (e.g., oncogenes that are overexpressed from an artificial promoter); (ii) in the majority of cases, they did not interrogate the most clinically relevant stages of disease (i.e., late-stage and/or metastatic disease); (iii) they failed to examine clinically relevant combinations of targeted agents with standard-of-care chemotherapeutics, particularly for front-line disease settings; and (iv) they often did not establish appropriate endpoints of therapeutic response/outcome, such as overall survival (OS) and progression-free survival (PFS). We recently conducted an in-depth series of preclinical case studies in which we assessed the translational use of 2 highly validated *Kras*-mutant GEMMs, one of non-small cell lung cancer (NSCLC) and one of pancreatic ductal

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adenocarcinoma [PDAC (9)]. In this study, we first defined several requirements for more-accurate simulation of human clinical trials in GEMMs, and then we examined combinations of chemotherapy and targeted therapeutics that have been tested in key historical and recent trials. We showed that GEMMs can indeed be used to recapitulate clinical trial data accurately. Of note, in the case of combinations of the EGFR inhibitor erlotinib and conventional chemotherapeutics, we reproduced not only the subtle OS impact of erlotinib in combination with gemcitabine observed in human pancreatic cancer (10) but also the currently unexplained negative interactions seen in a subset of patients with NSCLC harboring *KRAS* mutations (11). To simulate combination trials with the human-specific anti-VEGF antibody bevacizumab, we used a surrogate monoclonal antibody (B20-4.1.1) that binds murine VEGF with a comparable affinity (12). B20-4.1.1 treatment in combination with carboplatin in the NSCLC GEMM resulted in an OS improvement versus chemotherapy alone, similar to what was observed in the pivotal Eastern Cooperative Oncology Group trial (ECOG 4599) that led to the approval of bevacizumab for the treatment of NSCLC patients (13). However, the PDAC GEMM treated with the combination of gemcitabine and B20-4.1.1 showed a mixed survival response: approximately half of the mice showed no benefit in comparison with gemcitabine alone, whereas the other half showed improved survival. This was unlike the lack of any impact observed with the addition of bevacizumab to gemcitabine in the corresponding human trial [Cancer and Leukemia Group B (CALGB) 80303 (14)]. The dichotomous survival pattern we observed in the GEMM may be a reflection of tumor heterogeneity, especially considering the encouraging responses that were seen in earlier phase II clinical trials (15), and it underscores the need to better understand and define responsive patient subsets. In addition to OS, we attempted to model PFS based on noninvasive imaging data from each of the above-mentioned GEMM preclinical trials. To our knowledge, this was the first effort to assess this key endpoint in an animal model. Most of the PFS data were concordant with the observed OS patterns, although it was clear in some cases that the imaging intervals we used did not always allow for adequate resolution and could benefit from further refinement. Altogether, the results of this study attained the broad goal of establishing a unique role for GEMMs in the assessment of tumor therapies and laid the foundation for their preclinical use by showing their translatability via multiple relevant endpoints. It is also important to note that this study adhered to strict U.S. and international guidelines concerning animal welfare (16). Every model and preclinical study must be uniquely evaluated, and investigators must always work closely with their institutional animal care and use committee, as well as veterinary and animal-care staff, to appropriately define and follow humane endpoints (e.g., body weight, activity, and condition) and specialty care prior to study commencement.

The question now at hand is, How do we best leverage the lessons learned from this and other studies to improve the drug development process and success rate of human clinical trials? GEMMs can inform several key aspects of the clinical process, as summarized in Fig. 1. First, complex GEMMs have the potential to better reflect patient/tumor diversity within a given indication, thereby influencing patient selection through the discovery and validation of new predictive biomarkers. For example, a majority of pancreatic cancers (up to ~90%) show mutation of *KRAS* and exhibit concurrent inactivation of the *P16/CDKN2A* locus; additionally, *TP53* is commutated in a subset of these tumors [up to ~70% (17, 18)]. However, data in the literature regarding the *TP53* mutation as a prognostic indicator for this disease, and how it may influence the response to standard-of-care gemcitabine chemotherapy, are conflicting (19, 20). Of interest, Olive and colleagues (21) recently showed that the desmoplastic stroma could serve as a barrier to gemcitabine delivery in a *Trp53* mutant PDAC GEMM. Of note, this negative impact on drug delivery, and consequently efficacy, was unique to the spontaneous GEMM and was not observed in either subcutaneous or orthotopic implant models. Taken together, these data indicate that *TP53* status may serve as a predictive biomarker for treatment response in PDAC. This hypothesis can be tested with extant mutant *Kras*–driven pancreatic cancer GEMMs that represent all possible combinations of *Kras* mutation with *P16/Cdkn2A* and *Trp53* tumor suppressor inactivation (22). The availability of these GEMMs also makes it possible to interrogate p53 modulation of response to combinations of gemcitabine with novel agents, which could be particularly important for agents that modulate cell-cycle progression and/or apoptosis. Nevertheless, even with the ever-increasing repertoire of animal models that emulate key genetic driver mutations found in various human cancers, it is important to highlight that many GEMMs do not fully recapitulate all aspects of the clinical population, including the full extent of genetic diversity and metastatic spread (23). However, as a result of the differences between humans and mice, GEMMs provide a unique opportunity for both identifying and validating modifiers of tumor progression and therapeutic response. Because mice are inbred and inherently less genetically diverse than humans, investigators can use them to decipher which mutations are actually worth pursuing by cross-referencing tumors and/or tumor types driven by the same genetic alterations in both mice and humans (24). Moreover, the ability to have truly normal, strain-matched genetic controls in mice may prove invaluable for interpreting the relevance of thousands of genetic changes that are revealed upon whole-genome sequencing of human tumors and metastases (25, 26). As pharmacogenomic criteria become increasingly de rigueur for patient stratification in early clinical trials (27), GEMMs have the potential to both enable the investigation of basic aspects of tumor biology (which may not be possible in the clinic) and provide proof-of-concept for novel targeted agents and companion biomarkers.

GEMMs can also play a key role in elucidating the mechanisms of therapeutic response and innate resistance to both chemotherapy and targeted agents. Indeed, GEMMs have already been successfully applied in this manner to

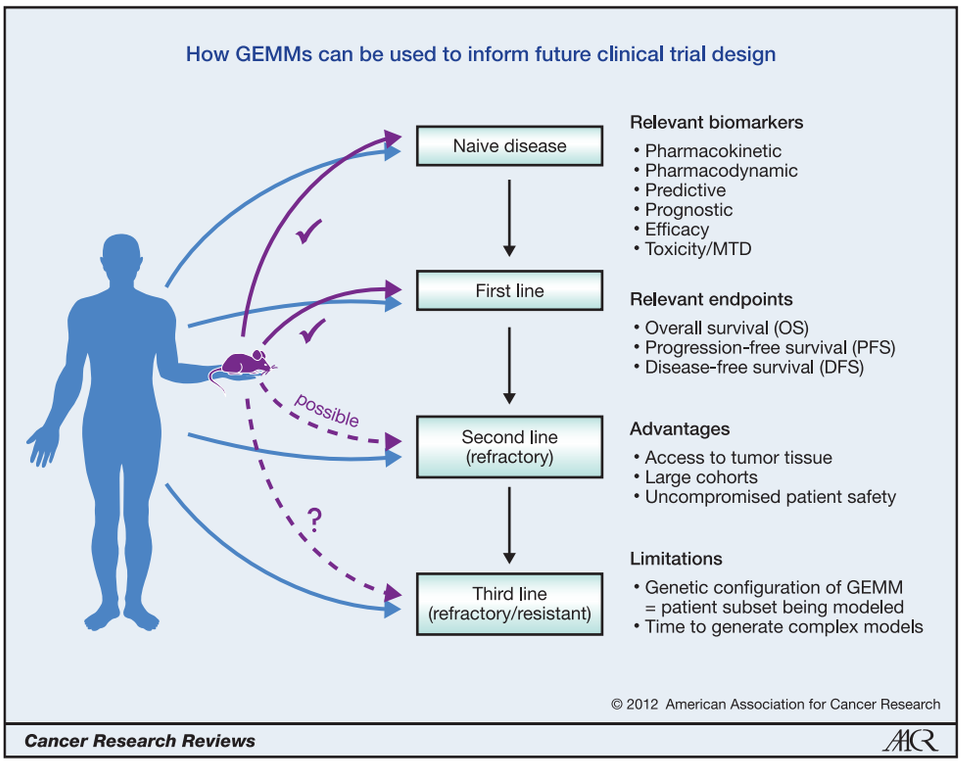

Figure 1. Use of GEMMs for preclinical development. The figure depicts the established route for human clinical trial development and which steps may be informed by GEMMs. These models can be used to further validate both known and newly discovered cancer targets and pathways. In addition, they may facilitate the identification and validation of relevant pharmacokinetic, pharmacodynamic, predictive, and prognostic biomarkers for evaluating drug efficacy, toxicity, and various survival endpoints. Although GEMMs may be limited to certain patient populations and subsets of disease, their overall use may be critical for informing first-line clinical trial design as well as refractory and resistant disease progression for better therapeutic identification and patient safety.

investigate intrinsic resistance to standard-of-care chemotherapeutic agents in acute myeloid leukemia (28). The notion that GEMMs may be able to enhance our understanding of inherent response versus resistance to targeted therapies is exemplified by the current debate on the use of *KRAS* mutations to stratify patients receiving EGFR inhibitors. Although it is clear that *RAS* mutations are drivers of poor prognosis in several cancers, the implementation of *KRAS* mutational status as a predictive biomarker is relatively recent and still controversial (29, 30). This is particularly important because adverse interactions resulting from EGFR inhibition in patients with *KRAS* mutant tumors have only been observed in a subset of trials within a given indication (NSCLC or colorectal cancer) and when these agents were combined with some (but not all) chemotherapeutics. Even in the latter case, the effects varied with the dosing regimen. For example, no deleterious effects were observed in NSCLC patients whose tumors harbored *KRAS* mutations when erlotinib was dosed sequentially (the SATURN study) versus concurrently [the TRIBUTE study (11, 31, 32)]. Furthermore, these effects could also depend on the tumor type, as the combination of erlotinib with gemcitabine resulted in both PFS and OS benefits in patients with pancreatic cancer, a population in which the majority of tumors harbor *KRAS* mutations (10). Hence, a more rigorous investigation and understanding of the mechanisms that influence *KRAS* mutant tumor responses, such as wild-type EGFR, ERBB3, cMET, and phosphoinositide 3-kinase (PI3K) pathway activation levels, is critically needed to better inform and guide patient stratification criteria based on *KRAS* mutational status (33–36). Given that GEMMs recapitulate these subtle differences, these models offer a means to systematically dissect the underlying mechanisms of interaction between EGFR inhibition and perturbation of RAS signaling in the context of therapeutic intervention, a vital issue that cannot be easily investigated via clinical trials alone. Along these lines, Zhou and colleagues (37) recently used nongermline GEMMs for NSCLC harboring *Her2*, *Egfr*, and *Kras* mutations to show differential activation of the PI3K/AKT and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling pathways in tumors with mutant *Her* receptor tyrosine kinases (RTK) compared with mutant *Kras* lesions, and they correlated the results with treatment response to a small-molecule EGFR inhibitor. Similarly, Engelman and colleagues (38) used a *Kras*-mutant NSCLC GEMM to examine the combinatorial efficacy of mitogen-activated protein–extracellular signal-regulated kinase (MEK) and PI3K inhibitors, a strategy that is now being pursued clinically. However, the modulation of these downstream pathways

by targeted therapy in either clinically relevant first-line (in combination with standard-of-care chemotherapy) or refractory settings still requires detailed investigation, as does the question of how these same pathway alterations modulate therapeutic response in other KRAS-mutant indications, such as PDAC.

Finally, the use of GEMMs may enable investigators to explore both the feasibility and validity of a personalized medicine approach. In two recent studies of patients with NSCLC, Kim and colleagues (39) and Sequist and colleagues (40) addressed the issue of whether we can tailor customized treatment regimens for patients based on their tumor's molecular profile as well as the evolution of resistance to targeted therapy. The landmark phase II BATTLE (Biomarker-integrated Approaches of Targeted Therapy for Lung Cancer Elimination) study (39) showed for the first time that tumor biopsies from previously treated patients with advanced NSCLC could be safely collected and effectively analyzed in real time for a large number of diverse biomarkers to facilitate personalized treatment. The results are encouraging and suggest that biomarker-driven patient stratification has the potential to increase overall treatment efficacy and response rates; however, this remains to be confirmed in larger phase III trials. The Bayesian trial design used in BATTLE allowed the investigators to observe correlations that might not have been predicted *a priori*. An intriguing example of this is the somewhat unexpected and promising relationship observed between *KRAS*- or *BRAF*-mutant disease and responsiveness to sorafenib. It seems highly likely that the trial design and adaptive randomization approach facilitated this particular observation, at least in part, by steering a preponderance of patients who were positive for the *KRAS/BRAF* biomarker signature toward the sorafenib arm and away from the 2 EGFR-inhibitor–containing arms. Currently, no targeted therapies have been specifically approved for *KRAS*-mutant NSCLC; therefore, it will be very interesting to determine whether this relationship will withstand further testing in NSCLC as well as other mutant *KRAS*–driven cancers. Moreover, it will be important to discern which mechanism(s) and cell population(s) targeted by sorafenib (e.g., tumor cell and/or endothelial cell compartment) mediate this efficacious response, particularly given that specific targeting of VEGF signaling is sufficient to provide highly significant improvements in both PFS and OS in the $Kras^{LSL\text{-}G12D}; p53^{frt/frt}$ NSCLC GEMM (9). Lastly, it will be interesting to see whether more selective and/or potent inhibitors of the VEGF/R2 and RAF/MEK/ERK signaling pathways, and combinations thereof, can provide an even greater response rate. The availability of well-validated, mutant *Kras*–driven GEMMs of NSCLC and PDAC gives investigators a valuable preclinical opportunity to interrogate each of these questions in a controlled and accessible manner.

In the second study, Sequist and colleagues (40) applied a serial tumor biopsy approach to examine the spectrum of alterations that arose in a small cohort of patients who had been subjected to targeted EGFR inhibitors as well as more conventional, follow-on treatment regimens. The authors identified both previously reported and novel alterations. Of importance, these studies show that real-time tumor biopsies from patients in clinical trials can yield invaluable information about the mechanisms of both therapeutic response and resistance. However, this approach can have significant practical limitations in terms of tumor accessibility, particularly for serial biopsies, as well as patient safety and availability (41). Although human tumor cell lines and explants provide additional systems for interrogating therapeutic response and resistance both *in vitro* and *in vivo* (42–44), they cannot mimic the complete array of interactions that occur among the diverse cellular players that constitute the innate tumor microenvironment and metastatic niche(s), or the contributions made by each player. As such, they can never reveal the full repertoire of alterations that mediate efficacy and/or acquired resistance that arises in response to therapy. Thus, GEMMs, with their ability to mimic therapeutic resistance *in toto* and increased tumor tissue accessibility, could provide an invaluable means to model therapeutic response, refractory patient populations, and the dynamic evolution of tumors on treatment. An excellent example of GEMMs applied in this fashion is found in the work of Politi and colleagues (45), who showed that mouse models of mutant EGFR–driven lung carcinomas give rise to drug-resistant tumors that exhibit the same molecular changes that confer resistance in human lung cancers when treated with the tyrosine kinase inhibitor erlotinib. Similarly, GEMMs have been successfully used to model acquired resistance to chemotherapy and have provided key insights into the underlying mechanisms and cell types involved. For example, a model of BRCA1-related breast cancer was used to show that even subtle alterations in the levels of a drug efflux transporter in tumor cells can confer resistance to doxorubicin (46, 47). In addition, Gilbert and Hemann (48) recently used a well-established mouse model of Burkitt's lymphoma to show that stromal factors released from thymic endothelial cells can create a chemoresistant niche that fosters lymphoma cell survival following chemotherapy and eventual tumor relapse. It is important to note that biomedical imaging and other readouts (e.g., circulating tumor cells and serum-based biomarkers) will be needed to facilitate preclinical efforts that are less amenable to real-time and serial tumor biopsies (e.g., GEMMs of lung or pancreatic cancer vs. skin, breast, or colorectal cancer).

In conclusion, recent preclinical studies in GEMMs have made substantial strides in modeling clinically relevant endpoints and making germane predictions regarding human responses as well as resistance to targeted therapies (and combinations thereof). In this era of molecular medicine, the ability to recapitulate clinical oncology scenarios in the appropriate microenvironmental context uniquely qualifies GEMMs as platforms for discovering predictive biomarkers, elucidating the determinants of clinical responses, and understanding the mechanisms by which tumors evolve resistance to increasingly complex therapeutic strategies.

Disclosure of Potential Conflicts of Interest

L. Johnson owns restricted stock units and stock options in Roche and is a member of the Scientific Working Group that advises the Center for Advanced Preclinical Research (CAPR) at the NCI and is compensated for travel expenses. M. Singh is an employee of Genentech/Roche and holds shares in Roche. C. Murriel is an employee of OncoMed Pharmaceuticals, Inc. and owns stock options in the company.

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