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 predominately, 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. 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 and, in particular, relevant stages of disease; and (iv) they often did not establish appropriate endpoints of therapeutic response/outcome, such as overall survival (OS) and progression-free survival (PFS).

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...
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 \( \text{KRAS} \) and exhibit concurrent inactivation of the \( \text{P16/CDKN2A} \) locus; additionally, \( \text{TP53} \) is commuted in a subset of these tumors [up to ~70% (17, 18)]. However, data in the literature regarding the \( \text{TP53} \) 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 \( \text{Trp53} \) mutant PDAC GEMM. Of note, this negative impact on drug delivery, and consequently efficacy, was unique to the spontaneous \( \text{GEMM} \) and was not observed in either subcutaneous or orthotopic implant models. Taken together, these data indicate that \( \text{TP53} \) status may serve as a predictive biomarker for treatment response in PDAC. This hypothesis can be tested with extant mutant \( \text{Kras} \)-driven pancreatic cancer GEMMs that represent all possible combinations of \( \text{Kras} \) mutation with \( \text{P16/CDkn2A} \) and \( \text{Trp53} \) tumor suppressor inactivation (22). The availability of these GEMMs also makes it possible to interrogate \( \text{p53} \) 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 pharmacogenic 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...
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 (MEK) 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 significant targeting of VEGF signaling is sufficient to provide highly significant improvements in both PFS and OS in the KRAS<sub>LSL-G12D;</sub>p53<sup>fl/fl</sup> 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 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. Murrzel is an employee of OncoMed Pharmaceuticals, Inc. and owns stock options in the company.

Acknowledgments

We thank Melissa Junttila for providing critical feedback and Allison Bruce for excellent graphics.

Received August 17, 2011; revised January 3, 2012; accepted January 16, 2012; published OnlineFirst May 16, 2012.

References

17. The Cancer Genome Project [homepage on the Internet]. Available from: http://www.sanger.ac.uk/genetics/CGP/
Genetically Engineered Mouse Models: Closing the Gap between Preclinical Data and Trial Outcomes

Mallika Singh, Christopher L. Murriel and Leisa Johnson

Cancer Res  Published OnlineFirst May 16, 2012.

Updated version  Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-11-2786