Adenocarcinomas are the most prevalent non-small cell lung cancers (NSCLC), accounting for ~50% of cases. Several therapeutically targetable mutant oncogenic kinases have been identified, including epidermal growth factor receptor (EGFR), HER2, and anaplastic lymphoma kinase (1). However, the overall prevalence and discovery of compensatory mutations has limited the clinical efficacy of agents targeting these kinases to relatively small subsets of patients. Among these other mutations, preexisting or acquired mutations of KRAS, which is downstream of these kinases, often renders adenocarcinomas resistant to targeted agents as well as standard therapies. Likewise, diverse mutations can result in loss or gain of function of tumor suppressors such as p53, which also variably affect responsiveness, and increase the complexity of effective targeting.

Such a diversity of mutations resulting in oncogene activation and loss of tumor suppressor genes can still lead to activation of common pathways that mediate cancer pathogenesis and therapeutic resistance. Nonmutant components of these essential pathways may be specifically targeted by molecular therapeutics, making them potential targets in cancers in which the multiplicity of driver and acquired mutations can make targeting individual mutants impractical. Previously, Meylan and colleagues (2) developed Kras-activated/p53-deficient mice in which they demonstrated that targeting individual mutants can make targeting individual mutants impractical. Among these other mutations, preexisting or acquired mutations of KRAS, which is downstream of these kinases, often renders adenocarcinomas resistant to targeted agents as well as standard therapies. Likewise, diverse mutations can result in loss or gain of function of tumor suppressors such as p53, which also variably affect responsiveness, and increase the complexity of effective targeting.

The NF-κB/REL family includes 5 homologous proteins, which form heterodimeric signal-activated transcription factors (3). A variety of physiologic and oncogenic signals promote inhibitor κB kinase (IKK)-mediated phosphorylation of inhibitor-κBs, which undergo proteasomal degradation, resulting in cytoplasmic-nuclear translocation and transactivation of NF-κBs (Fig. 1). Under current paradigms, the IKKs and NF-κBs signal via canonical and noncanonical pathways. Activation of the canonical IKK-NF-κB pathway (Fig. 1A), which has been most extensively studied, regulates multiple prosurvival genes that determine the malignant phenotype and resistance to therapy in a variety of cancers. Mutations that can activate this pathway in adenocarcinomas include EGFR, HER2, and KRAS. In addition, inactivation of p53 enhances NF-κB transactivation (Fig. 1B). The noncanonical pathway activated by LTβ is important in lymphomas and may play a greater role in carcinomas than appreciated. Proteasome and IKKβ inhibitors that block NF-κB activation, as well as inhibit tumorigenesis and therapeutic resistance to cytotoxic therapies, have been developed (3).

In this issue of Cancer Discovery, Xue et al. (4) show both the promise and the possible limitations of inhibitors targeting NF-κB activation in adenocarcinomas of Kras-activated/p53-deficient mice. They discuss the ability of the Food and Drug Administration–approved proteasome inhibitor bortezomib to inhibit nuclear activation of canonical member Rel A(p65), repress NF-κB target genes, and induce cell death in murine adenocarcinoma lines derived from these mice. Bortezomib also induced regression of lung tumors and prolonged survival in Kras-activated/p53-deficient mice, but not of tumors from Kras-activated/wild-type p53 mice. Tumors from the double mutant mice also exhibited greater NF-κB inhibition in the absence of p53, suggesting that increased NF-κB and decreased p53 confer sensitivity. However, these initially sensitive lung tumors became resistant to bortezomib. The authors found similar response and acquired resistance in an orthotopic transplant tumor model. This resistance was not due to changes in NF-κB/Rel family member expression or to proteasome inhibitor resistance that altered sensitivity to NF-κB inhibition, indicating that alternative mechanisms mediate resistance. Similar therapeutic efficacy and acquired resistance were observed with IKK inhibitor Bay 117082, which is a more specific NF-κB inhibitor, reducing the likelihood that results were due to unrelated effects of proteasome inhibition. These findings highlight the potential of mouse models that recapitulate genetic alterations found in human tumors, such as lung adenocarcinomas, to exhibit potential sensitivity and resistance to targeted agents, as well as the mechanisms for these effects in vivo.

A caveat regarding genetically defined murine tumor models is the possibility they will predict greater sensitivity than will be found in human tumors in clinical trials. Consistent complexity of effective targeting.

**Author’s Affiliation:** Head and Neck Surgery Branch, National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Bethesda, Maryland

**Corresponding Author:** Carter Van Waes, Clinical Research Center, Room 4-2732, 10 Center Drive, National Institutes of Health, Bethesda, MD 20892. Phone: 301-402-4216; Fax: 301-402-1140; E-mail: vanwaesc@nih.gov

doi:10.1158/2159-8290.CD-11-0159

©2011 American Association for Cancer Research.
members NF-κB2/RELB were unaffected (6). Although the proteasome was previously implicated in activation of both pathways, this finding suggested that differential sensitivity and compensatory effects of these other REL/NF-κB family members could contribute to resistance. Further studies support novel roles for c-REL and RELB in the malignant phenotype of SCC (ref. 7; L. Nottingham and C. Van Waes, unpublished observations). Moreover, bortezomib did not inhibit other coactivated prosurvival transcription factors, such as AP-1 or STAT3 (6). NF-κB and these transcription factors have been demonstrated to coregulate expression of overlapping and distinct gene programs that promote the malignant phenotype and resistance of SCC. Supporting this, combination of bortezomib with either JNK or STAT3 inhibitors was shown to overcome bortezomib resistance (8, 9). Combining bortezomib with EGFR inhibition upstream of these pathways also enhanced activity in preclinical studies, but, when combined with radiation in a phase I trial, was unexpectedly associated with early disease progression (10). Antagonism of radiation-induced EGFR degradation with coactivation of

**Figure 1.** NF-κB activation in pathogenesis and therapy of cancer. A, canonical NF-κB activation is inducible by chronic exposure to bacteria, certain viral products, chemical promoters, carcinogens, cytotoxic agents, and reactive oxygen species (ROS). Repeated DNA damage may result in constitutive activation of oncopgenes upstream of the IKK complex, including EGFR, Her2, and KRAS. Intermediate kinases convey signals to the inhibitor-κB complex formed by IKKα, β, and γ, and IKKβ and CK2 phosphorylate inhibitor-κB, marking it for ubiquitination and proteasome degradation. P105/RELA or cREL is processed to NF-κB1 (p50)/RELA or cREL heterodimers, which translocate to the nucleus and bind promoters of genes regulating proliferation, apoptosis, migration, inflammation, angiogenesis, and innate immunity. B, inactivation of tumor suppressor p53 enhances NF-κB activation. C, noncanonical pathway. The alternative pathway may be activated by other TNF family members via NIK and involves IKKα/IKKβ homodimers, which activate NF-κB2/p100 for processing into p52/RelB heterodimers. The Rel-B/p52 heterodimer then translocates into the nucleus to bind the promoters of genes whose products are important for the malignant phenotype in some cancers and B-cell development and adaptive immunity. Red-highlighted activators in adenocarcinoma include EGFR, Her2, KRAS; inhibitors of NF-κB activation under clinical investigation include proteasome and IKK antagonists; CK2, casein kinase 2; EGFR, epidermal growth factor receptor; FAK, focal adhesion kinase; IL-1R, interleukin-1R; NIK, NF-κB-inducing kinase; TAK, TGF-β–activated kinase; TNFR, TNF receptor; TRAF, TNF receptor–associated factor; PDK, 3-phosphoinositide–dependent protein kinase; PI3-K, phosphatidylinositol 3-kinase.

with this, response rates of <10% and limited duration of response to bortezomib as a single agent or in combination with docetaxel were observed in a phase II trial of NSCLC (5). These findings could not be attributed solely to inclusion of other histologic types, as >50% were adenocarcinomas. Why is this so? Adenocarcinomas and other human solid malignancies often exhibit a greater accumulation of mutations that reflect prolonged carcinogen exposure and genomic instability prior to diagnosis (1). Thus, elucidation of the basis for proteasome and IKK inhibitor resistance in lung adenocarcinomas has important implications regarding future uses for these agents.

Important clues regarding possible mechanisms for proteasome inhibitor resistance in cancer have been obtained from squamous cell carcinomas (SCC), which constitute another major histologic subset of lung and head and neck cancers. Interestingly, in a phase I study of bortezomib in head and neck cancers, inhibitory effects on activation of nuclear p65 and target genes were observed, but nuclear activation of the canonical member, c-REL, and noncanonical members NF-κB2/RELB were unaffected (6). Although the proteasome was previously implicated in activation of both pathways, this finding suggested that differential sensitivity and compensatory effects of these other REL/NF-κB family members could contribute to resistance. Further studies support novel roles for c-REL and RELB in the malignant phenotype of SCC (ref. 7; L. Nottingham and C. Van Waes, unpublished observations). Moreover, bortezomib did not inhibit other coactivated prosurvival transcription factors, such as AP-1 or STAT3 (6). NF-κB and these transcription factors have been demonstrated to coregulate expression of overlapping and distinct gene programs that promote the malignant phenotype and resistance of SCC. Supporting this, combination of bortezomib with either NIK or STAT3 inhibitors was shown to overcome bortezomib resistance (8, 9). Combining bortezomib with EGFR inhibition upstream of these pathways also enhanced activity in preclinical studies, but, when combined with radiation in a phase I trial, was unexpectedly associated with early disease progression (10). Antagonism of radiation-induced EGFR degradation with coactivation of
NF-κB and these other prosurvival pathways was observed, providing a cautionary tale about the limits of extrapolation from experimental models.

Despite potential limitations, the models reported by the Jacks group will likely be useful in studying the mechanisms of acquired resistance to proteasome and IKK inhibitors, as well as identifying active combinations for testing in clinical trials in patients with lung adenocarcinomas. In addition to the ability to perform mechanistic studies, these cancers are relatively less accessible in patients, increasing the importance of mouse models for determining mechanisms of resistance. Still uncertain is the feasibility of sustained inhibition of NF-κB by the proteasome and IKKs in therapy of cancer. The roles of these molecules in homeostasis, injury, and infection are complex and may be essential for cell and organismal survival (3). It is hoped that combinations with inhibitors of other pathways that preferentially sensitize cancer cells without unacceptable toxicities will be defined.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Published online August 16, 2011.

REFERENCES

Targeting NF-κB in Mouse Models of Lung Adenocarcinoma

Carter Van Waes