Published in final edited form as:

Cancer Discov. 2011 August ; 1(3): 200–202. doi:10.1158/2159-8290.CD-11-0159.

Targeting Nuclear Factor- κ B in mouse models of lung adenocarcinoma

Carter Van Waes

Head and Neck Surgery Branch, National Institute on Deafness and Other Communication Disorders, National Institutes of Health, Bethesda, Maryland

Summary

Xue et al demonstrate response and increased survival but development of acquired resistance to proteasome and Inhibitor- κ B Kinase inhibitors targeting NF- κ B activation in adenocarcinomas of Kras-activated, p53 deficient mice

Adenocarcinomas are the most prevalent non-small cell lung cancers (NSCLCs), 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. Non-mutant components of these essential pathways may be specifically targeted by molecular therapeutics, making them potential targets in cancers where the multiplicity of driver and acquired mutations can make targeting individual mutants impractical. Previously, Jacks and colleagues developed Kras-activated/p53-deficient mice in which they demonstrated increased development of lung adenocarcinomas that required activation of NF- κ B signaling (Fig. 1) (2).

The NF- κ B/REL family includes 5 homologous proteins, which form heterodimeric signal activated transcription factors (3). A variety of physiologic and oncogenic signals promote Inhibitor κ B kinase (IKK) mediated phosphorylation of Inhibitor- κ Bs, which undergo proteasomal degradation, resulting in cytoplasmic-nuclear translocation and transactivation of NF- κ Bs (Fig. 1). Under current paradigms, the IKKs and NF- κ Bs signal via canonical and non-canonical pathways. Activation of the canonical IKK-NF- κ B pathway (Fig. 1A) has been most extensively studied, and regulates multiple prosurvival genes that determine the malignant phenotype and resistance to therapy in a variety of cancers. Mutations that activate this pathway in adenocarcinomas include EGFR, HER2, and KRAS. In addition, inactivation of p53 enhances NF- κ B transactivation (Fig. 1B). The non-canonical pathway, activated by LT β is important in lymphomas and may play a greater role in carcinomas than

appreciated. Proteasome and IKK β inhibitors have been developed which block NF- κ B activation, and inhibit tumorigenesis and therapeutic resistance to cytotoxic therapies (3).

In this issue, Xue et al., demonstrate both the promise and possible limitations of inhibitors targeting NF- κ B activation in adenocarcinomas of Kras-activated/p53-deficient mice (4). They demonstrate the ability of the FDA-approved proteasome inhibitor Bortezomib to inhibit nuclear activation of canonical member Rel A(p65), repress NF- κ B 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- κ B in the absence of p53, suggesting that increased NF- κ B 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 changes in NF- κ B/Rel family member expression, or proteasome inhibitor resistance that altered sensitivity to NF- κ B inhibition, indicating that alternative mechanisms mediate resistance. Similar therapeutic efficacy and acquired resistance was observed with IKK inhibitor Bay 117082, which is a more specific NF- κ B inhibitor, reducing the likelihood that the results were due to unrelated effects of proteasome inhibition. These results highlight the potential of mouse models that recapitulate genetic alterations found in human tumors such as lung adenocarcinomas, to demonstrate potential sensitivity and resistance to targeted agents, and 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 with this, response rates of less than 10% and limited duration of response to Bortezomib as a single agent or in combination with docetaxel was observed in a phase II trial of NSCLC (5). This could not be attributed solely to inclusion of other histologic types, as over 50% were adenocarcinomas. Why is this? 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 the canonical member, c-REL, and non-canonical members NF- κ B2/RELB were unaffected (6). Although the proteasome was previously implicated in activation of both pathways, this suggested that differential sensitivity and compensatory effects of these other REL/NF- κ B family members could contribute to resistance. Further studies support novel roles for c-REL and RELB to the malignant phenotype of SCC (7; L. Nottingham and C. Van Waes, unpublished observations). Moreover, bortezomib did not inhibit other co-activated prosurvival transcription factors, such as AP-1 or STAT3 (6). NF- κ B and these transcription factors have been demonstrated to co-regulate 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). Combination of 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 co-

activation of NF- κ B 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 study of the mechanisms of acquired resistance to proteasome and IKK inhibitors, and identification of 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- κ B by the proteasome and IKKs in therapy of cancer. The role of these molecules in homeostasis, injury, and infection are complex and may be essential for cell and organismal survival (3). Hopefully combinations with inhibitors of other pathways will be defined which preferentially sensitize cancer cells without unacceptable toxicities.

REFERENCES

1. Pao W, Iafrate AJ, Su Z. Genetically informed lung cancer medicine. *J. Pathol.* 2011; 223:230–40. [PubMed: 21125677]
2. Meylan E, Dooley AL, Feldser DM, Shen L, Turk E, Ouyang C, et al. Requirement for NF-kappaB signaling in a mouse model of lung adenocarcinoma. *Nature.* 2009; 462:104–7. [PubMed: 19847165]
3. Van Waes C. Nuclear Factor-kappaB in development, prevention and therapy of head and neck cancer. *Clin Cancer Res.* 2007; 13:1076–82. [PubMed: 17317814]
4. Xu W, Meylan E, Oliver TG, Feldser DM, Winslow MM, Bronson R, Jacks T. Response and resistance to NF-kB inhibitors in mouse models of lung adenocarcinoma. *Cancer Disc.* 2011
5. Fanucchi MP, Fossella FV, Belt R, Natale R, Fidias P, Carbone DP, et al. Randomized phase II study of bortezomib alone and bortezomib in combination with docetaxel in previously treated advanced non-small cell lung cancer. *J Clin Oncol.* 2006; 24:5025–33. [PubMed: 17075122]
6. Allen C, Saigal K, Nottingham L, Arun P, Van Waes C. Bortezomib-induced apoptosis with limited clinical response is accompanied by inhibition of canonical but not alternative NF-kappaB subunits in head and neck cancer. *Clin Cancer Res.* 2008; 14:4175–85. [PubMed: 18593997]
7. Yang X, Lu H, Yan B, Roman RA, Bian Y, Friedman J, et al. DeltaNp63 versatily regulates a broad NF-kappaB gene program and promotes squamous epithelial proliferation, migration and inflammation. *Cancer Res.* 2011; 71:3688–3700. [PubMed: 21576089]
8. Chen Z, Ricker JL, Malhotra PS, Nottingham L, Arun P, Chen Z, Van Waes C. Differential bortezomib sensitivity in headand neck cancer corresponds to proteasome, nuclear factor-kappaB and activator protein-1 related mechanisms. *Mol Cancer Ther.* 2008; 7:1949–60. [PubMed: 18645005]
9. Li C, Zang Y, Sen M, Leeman-Neill RJ, Man DS, Grandis JR, Johnson DE. Bortezomib up-regulates signal transducer and activator of transcription-3 and synergizes with inhibitors of signal transducer and activator of transcription-3 to promote head and neck squamous cell carcinoma cell death. *Mol Cancer Ther.* 2009; 8:2211–20. [PubMed: 19638453]
10. Van Waes C, Duffy AG, Simone NL, Gibson MK, Arai Y, Rudy SF, et al. Early disease progression in a phase I study of bortezomib, cetuximab, and intensity-modulated radiation therapy for squamous cell carcinoma of the head and neck. *Clin Cancer Res.* 2011 in press.

Figure 1.

NF- κ B activation in pathogenesis and therapy of cancer. A. Canonical NF- κ B 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 oncogenes upstream of the IKK complex, including EGFR, Her2, and KRAS. Intermediate kinases convey signals to the Inhibitor- κ B complex formed by IKK α , β and γ , and IKK β and CK2 phosphorylate Inhibitor- κ B, marking it for ubiquitination and proteasome degradation. P105/RELA or cREL is processed to NF- κ B1 (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- κ B activation. C. Non-canonical Pathway. The alternative pathway may be activated by other TNF family members via NIK, and involves IKK α /IKK α homodimers, which activate NF- κ B2/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- κ B activation under clinical investigation include proteasome and IKK antagonists. Abbreviations: NF- κ B, nuclear factor κ B; IKK, Inhibitor- κ B kinase; NIK, NF- κ B inducing kinase; TNFR, Tumor Necrosis Factor Receptor; IL-1R, Interleukin 1 R; EGFR, Epidermal Growth Factor Receptor; TRAF, TNF receptor associated factor; TAK, transforming growth factor-beta activated kinase; FAK, Focal Adhesion Kinase; CK2, casein kinase 2; PI3-K, phosphatidylinositol 3-kinase; PDK, 3-phosphoinositide dependent protein kinase