tandem with resistance inhibitors is appropriate in an adjuvant setting or in an induction setting will be important issues to address. The National Cancer Institute's ALCHEMIST (Adjuvant Lung Cancer Enrichment Marker Identification and Sequencing Trial) trial only uses the first-generation TKI erlotinib in EGFR-mutant patients. Finally, at present, immunotherapy approaches are not effective in patients with EGFR mutations. Whether programmed cell death protein 1/programmed death-ligand 1 expression and tumor mutational burden are increased when NR2B is upregulated will be an interesting question.

In summary, this is a very interesting study that presents a novel resistance mechanism of EGFR TKI resistance. Elegant signal transduction experiments are described that may eventually lead to therapeutic

advances. Surgeons must be involved in and aware of this type of research so that we can develop new therapies for these patients and communicate effectively with oncologists and patients.

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Commentary: Keeping the target painted for precision cancer therapy

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Observations that activating mutations in the *epidermal growth factor receptor (EGFR)* gene confer sensitivity to EGFR-tyrosine kinase inhibitors (EGFR-TKIs) such as gefitinib and erlotinib ushered in the modern era of targeted therapy for non-small cell lung cancer (NSCLC).^{1,2} EGFR mutations occur in approximately 20% of NSCLC, typically in female patients, nonsmokers, and patients

CENTRAL MESSAGE

Acquired resistance to EGFR-TKI in NSCLC is associated with up-regulation of NRP2b. Targeting NRP2b may be a novel strategy to restore and maintain sensitivity to these agents for lung cancer therapy.

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with adenocarcinomas.³ Despite impressive initial responses, virtually all patients develop resistance to EGFR-TKI due to secondary mutations in *EGFR*, mutations that activate survival signaling pathways such as *MET*, evolution of a small cell lung cancer phenotype, or epithelial-to-mesenchymal transition (EMT).⁴ As such, delineation of mechanisms conferring resistance to EGFR-TKI is a major focus of investigative efforts in thoracic oncology.

In this issue of the *Journal*, Dimou and colleagues⁵ examined the role of *Neuropilin-2b (NRP2b)* in acquired resistance to EGFR-TKI in NSCLC. Previous studies by this

group^{6,7} had demonstrated that EMT induced in NSCLC cells by transforming growth factor beta (TGF- β) coincides with up-regulation of *NRP2b*, and that NRP2b is essential for TGF- β -mediated lung cancer progression and metastasis.⁷ Additional studies demonstrated that acquired resistance to EGFR-TKI coincides with and is dependent on up-regulation of *NRP2b*.⁷ Expression levels of *NRP2b* in primary lung cancers correlated with advanced stage of disease, programmed death-ligand 1 expression, and EMT phenotype. Notably, *NRP2b* expression was not detected in normal lung tissues.⁷

In the current study, Dimou and colleagues⁵ extended the aforementioned studies and observed that the development of resistance to gefitinib and osimertinib coincided with up-regulation of *NRP2b*. Knock-down of *NRP2b* virtually abolished outgrowth of resistant (persister) colonies in NSCLC with activating *EGFR* mutations following prolonged exposure to gefitinib or osimertinib; in contrast, knock-down of the closely related *NRP2a* isoform increased persister colony outgrowth. Interestingly, knock-down of *NRP2b* sensitized 1 of 2 NSCLC lines with *wt EGFR* to EGFR-TKI, raising the possibility that strategies to inhibit *NRP2b* expression/activity might translate to a subset of NSCLC without activating *EGFR* mutations.

In additional experiments, the authors demonstrated that NRP2b directly interacts with the multifunctional protein kinase glycogen synthase kinase 3 beta (GSK3 β) to induce phosphorylation of phosphatase and tensin homolog (PTEN), resulting in ubiquitination and proteosomal degradation of this tumor suppressor, and activation of protein kinase B (AKT) survival signaling. Whereas treatment with the GSK3 β inhibitor TDZD8 did not inhibit outgrowth of control lung cancer cells following chronic exposure to gefitinib (a first-generation EGFR-TKI), it did decrease outgrowth of these cells following exposure to the second-generation EGFR-TKI, osimertinib. The inhibitory effects of TDZD8 were pronounced in EGFR-TKI-resistant *NRP2a* knock-down NSCLC cells expressing only *NRP2b*.

Despite these provocative findings which demonstrate a novel mechanism of resistance to EGFR-TKI in NSCLC, the study is limited by the small number of cell lines used for the assays, and the fact that none of the in vitro

observations were validated with in vivo experiments. Moving forward, it will be important to examine a large panel of cell lines and tumor specimens to determine whether *NRP2b* expression levels correlate with *EGFR* mutation status and intrinsic or acquired resistance to EGFR-TKI. More extensive studies are necessary to determine the percentage of *wt EGFR* NSCLC that can be rendered sensitive to EGFR-TKI by inhibition of NRP2b activity. Although the use of GSK3 β inhibitors to reverse acquired resistance to EGFR-TKI is appealing, clinical development of these inhibitors has been futile despite nearly 20 years of intense efforts.^{8,9} In vivo experiments are necessary to determine on-target toxicities in normal tissues that could impact translation of these findings to the clinic. Despite these limitations, the authors are to be congratulated for the quality of their science and their methodic approach to a very complex, and highly relevant issue in thoracic oncology. Their work highlights the talent and creativity within our subspecialty. Inhibiting expression/activity of *NRP2b* may help “keep the target painted” for additional precision strikes against EGFR signaling in NSCLC.

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