untreated advanced *ALK*-positive NSCLC,[4] the current case was intrinsically refractory to crizotinib. Reportedly, EML4-ALK variants have different sensitivities to crizotinib, which correlates with differences in protein stability in EML4-ALK–expressing cells.[5] The significance of *ALK* exon 18 fusion variants observed in our case is currently unknown, but interestingly, the ALK transmembrane domain (Fig. 1*H*) is expressed, unlike in *ALK* exon 20 fusion variants. The transmembrane domain in exon 18–containing variants may be related to the differences in protein stability and resistance to crizotinib, although further studies—including of the function and subcellular localization of this variant—are necessary. For diagnosis of *ALK*-translocated lung cancer, screening by immunohistochemical analysis and verification by fluorescence in situ hybridization are currently recommended for convenience and cost. However, our case suggests that *ALK* fusion variants, except for exon 20 fusion variants, have a different therapeutic response to ALK inhibitors, although further studies are required for our variant. The guidance for *ALK* gene testing in patients with lung cancer by the Japan Lung Cancer Society also suggests that RT-PCR can provide reliable verification of *ALK* fusion status if chimeric transcripts can be verified by direct base determination, and ALK inhibitors can be indicated on the basis of this verification. In fact, we experienced an 80% response rate (four of five patients) for crizotinib in the *ALK*-positive patients who were identified only by RT-PCR because of technical problems. Therefore, use of RT-PCR may be a reasonable precaution to avoid overlooking some unusual *ALK* variant, especially when we cannot examine it by immunohistochemical analysis or fluorescence in situ hybridization. Transcript-based approaches and sequencing of ALK fusion variants can also optimize selection of ALK inhibitors for these patients.

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Prognostic and Predictive Value of *KRAS* Mutation in NSCLC

To the Editor:

With great interest, we have read the article by Zer et al.[1] regarding the prognostic effect of *KRAS* mutation in NSCLC. They conducted a pooled analysis to investigate the role of *KRAS* mutation subtypes in patients treated with epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKIs). Overall, *KRAS* mutation was not associated with prognostic and predictive benefit; however, these types of *KRAS* mutation were associated with different prognostic outcomes. In the placebo arms, patients with G12C/V mutations showed a favorable prognosis compared with those with G12D/S or G12A/R mutations (with median overall survival values: 6.3, 1.8, and 3.9 months, respectively; $p = 0.01$). When compared with the placebo group, EGFR TKIs significantly improved survival only in patients with G12D/S mutations (hazard ratio = 0.49, 95% confidence interval: 0.24–1.00, $p = 0.05$). Although these differences may be caused by activation of different downstream pathways (e.g., Akt and MEK),[2] we suggest another biological pathway that is associated with *EGFR*/*KRAS* signaling.

Liver kinase B1 gene (*LKB1*), a serine/threonine kinase, is a frequently mutated tumor suppressor gene in NSCLC. It regulates multiple cellular functions, such as cell bioenergetics metabolism, cell cycle arrest, and apoptosis. Despite significant association with *KRAS* mutation,[3] the relationship between liver kinase B1 (*LKB1*) and *KRAS* subtype has not been well understood. Nevertheless, *LKB1*-deficient *KRAS*-mutant

Disclosure: The authors declare no conflict of interest.

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ISSN: 1556-0864
http://dx.doi.org/10.1016/j.jtho.2016.04.035

(G12D) tumor shows a more invasive and metastatic phenotype with poorer prognosis than *KRAS* mutation alone in a mouse model of *KRAS*-induced NSCLC.[4] This finding suggests that patients with G12D/S may have poor prognosis owing to concurrent *LKB1* mutation.

Moreover, the presence of concurrent *LKB1* mutations may also account for treatment efficacy of EGFR TKIs. *LKB1* deficiency leads to enhanced mitochondrial metabolism that is associated with activation of adenosine monophosphoate–activated protein kinase and suppression of mammalian target of rapamycin signaling, resulting in increased sensitivity to EGFR TKI treatment without mutationally activated EGFR.[5] The authors showed that *LKB1*-mutant EGFR wild-type (WT) cell lines are more sensitive to erlotinib than are *LKB1*/*EGFR* WT cell lines. Overexpression of *LKB1* into *LKB1*-null A549 cells results in increased basal phosphorylation of adenosine monophosphoate–activated protein kinase subunit α and causes resistance to erlotinib. Furthermore, stable silencing of *LKB1* in *LKB1* WT cells increased sensitivity to erlotinib and resulted in less colony formation compared with the vector control. This suggests that *LKB1* status may serve as a predictive marker for patients with *KRAS* mutation when they are treated with EGFR TKIs.

The prognostic value of *KRAS* mutation has been discussed in many trials for decades, yet its value remains unclear. Further understanding of the correlation between *KRAS* subtype and *LKB1* mutation might be helpful in selecting patients who may benefit from EGFR TKIs.

The presence of *KRAS* mutation in colorectal cancer is indicative of nonsensitivity to anti-EGFR antibody therapy; therefore, its use is now part of routine oncology practice. Even so, the determination of *KRAS* status in the management of NSCLC and other malignancies is not considered standard practice on account of the lack of any convincing evidence that it is a predictive or prognostic marker. Although its role in clinical oncology has not been defined, preclinical and clinical findings suggest that activated mitochondrial signaling induced by *LKB1* mutation warrants further investigation in future studies.

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Response to Yamamoto et al.

In Response:

We thank Yamamoto et al.[1] for their comments on our study.[2] In their letter they suggest that co-mutations of liver kinase B1 gene (*LKB1*) and *KRAS* G12D may account for the differential prognostic and predictive effect we have reported for *KRAS* transition mutations G12D/G12S and the potential benefit from EGFR tyrosine kinase inhibitors in this subtype.

In recent years data has emerged suggesting that *KRAS* subtypes in NSCLC are not homogenous with respect to tumor phenotype,[3] prognosis,[4] and differential treatment effect of chemotherapy[5] or tyrosine kinase inhibitors.[1,6,7]

LKB1 (also known as serine/threonine kinase 11 gene [*STK11*]) is the third most frequently mutated gene in NSCLC (after tumor protein p53 gene [*TP53*] and *KRAS*) and is detected in approximately 20% of all lung adenocarcinoma.[8] The prognostic role of *LKB1* loss in NSCLC is controversial.[9–12] Previously reported studies suggest that *KRAS*-Mut/*LKB1*-Mut tumors differ in their response to mitogen-activated protein kinase and mammalian target of rapamycin signaling inhibition[13] and are resistant to docetaxel with or without selumetinib.[14] In fact, Skoulidis et al. recently provided

Disclosure: The authors declare no conflict of interest.

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ISSN: 1556-0864
http://dx.doi.org/10.1016/j.jtho.2016.08.130