

Primary Resistance to Immune Checkpoint Blockade in an *STK11/TP53/KRAS*-Mutant Lung Adenocarcinoma with High PD-L1 Expression

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Abstract: Several studies have shown that *STK11* and *TP53* mutations have different effects on the susceptibility to immune checkpoint blockade in *KRAS*-mutant non-small cell lung cancer (NSCLC). However, the impact of *STK11/TP53* co-mutations on treatment outcomes in the same clinical setting has never been reported. We recently encountered a case of a 70-year-old man who was diagnosed with advanced lung adenocarcinoma with high-programmed death-ligand 1 (PD-L1) expression. He received pembrolizumab monotherapy as a frontline treatment; however, the tumor did not respond to this therapy and showed deleterious outcome. Next-generation sequencing revealed that the tumor harbored a rare *STK11/TP53/KRAS* triple mutation. Our case suggests that these compound mutations may constitute a distinct, aggressive subset that is resistant to immunotherapy even when the tumor strongly expresses PD-L1. In addition, this report highlights the importance of using molecular profiling to detect co-mutations that can be associated with primary resistance or disease progression to improve survival even in the immunotherapy setting.

Keywords: lung cancer, *KRAS*, *STK11*, *TP53*, immune checkpoint inhibitors, prognosis

Introduction

KRAS mutations are the most prevalent oncogenic driver of non-small cell lung cancer (NSCLC), accounting for 20–30% of the lung adenocarcinoma cases.¹ They are associated with cigarette smoking and have been linked to poor prognosis without available targeted therapy.² The emergence of immune checkpoint inhibitors (ICIs) in recent years has enriched treatment options for NSCLC; however, clinical data on their efficacy in *KRAS*-mutant tumors has been inconsistent across different clinical settings.^{3,4} A recent study demonstrated that the treatment outcomes with ICIs were similar between *KRAS*-mutant and *KRAS*-wild type NSCLC and the response was better in programmed death-ligand 1 (PD-L1)-positive patients in both populations.⁵ Interestingly, evidence suggests that concurrent genomic alterations can affect the response and prognosis of *KRAS*-mutant NSCLC.^{6–9} Concurrent mutations in *STK11* have been associated with reduced survival in patients with *KRAS*-mutant tumors after chemotherapy.^{6–8} La Fleur et al evaluated the mutational status of 82 genes using targeted sequencing in 352 patients with NSCLC and demonstrated that *STK11* and *TP53* mutations were linked to poor patient survival in *KRAS*-mutant subtypes.⁹ These studies suggest that it is not *KRAS* mutations themselves, but co-mutations in other genes that

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determine the aggressive behavior of tumors and treatment outcomes in patients with the driving mutation.

Previous studies have shown that *STK11* and *TP53* mutations seldom overlap in treatment-naïve NSCLC patients,^{7,10} and the impact of these compound mutations on the efficacy of immunotherapy for *KRAS*-mutant NSCLC remains to be elucidated. Here, we report a case of lung adenocarcinoma harboring an *STK11/TP53/KRAS* triple mutation which showed poor response to first-line pembrolizumab monotherapy even though the tumor strongly expressed PD-L1.

Case Presentation

A 70-year-old male presented at our institution in September 2019 with a complaint of a persistent cough that had lasted for three months. He never smoked and had been taking statin and anti-hypertensive drugs for several years. His vital signs were stable and physical examination revealed no significant findings. Chest computed tomographic (CT) scan and positron emission tomography (PET) revealed a 4.8 cm x 4.3 cm-sized malignant lung

mass in the left lower lobe (LLL) invading the corresponding bronchus, and multiple mediastinal lymph nodes and bone metastases suggesting that the cancer was at stage IV (Figure 1A and B). Bronchoscopy showed irregular mucosal changes and a protruding mass at the orifice of the LLL, and histopathology revealed invasive mucinous lung adenocarcinoma positive for thyroid transcription factor 1 (TTF-1). Although routine mutation profiling ruled out several genetic alterations, including epidermal growth factor receptor mutations and anaplastic lymphoma kinase (*ALK*) or ROS proto-oncogene 1 (*ROS1*) fusions, the PD-L1 tumor proportion score (TPS), evaluated using the 22C3 pharmDx assay, was 100%. We initiated first-line pembrolizumab monotherapy (200 mg intravenously administered every three weeks) as a first-line treatment, expecting a favorable response. The first response evaluation conducted in November 2019 showed slight increase in the size of primary tumor mass and the mediastinal lymph nodes (Figure 1C). Taking the possibility of pseudoprogression into account, we continued with an

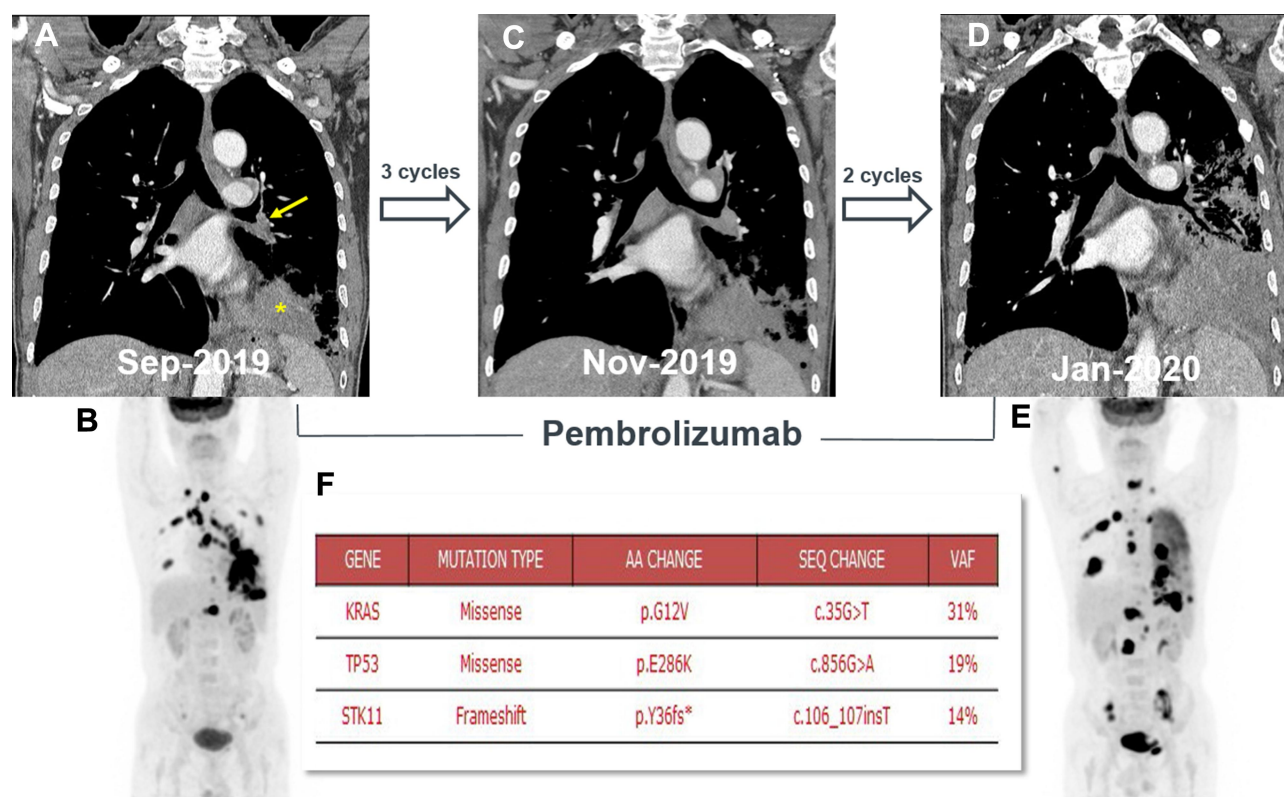


Figure 1 Chest computed tomographic (CT) and positron emission tomography (PET) scans performed during pembrolizumab treatment. (A) Initial chest CT scan showed a consolidative mass (asterisk) and a small endobronchial mass in the left lower lobe (LLL, arrow), which was later identified as invasive mucinous adenocarcinoma. (B) Baseline PET scan showed hypermetabolic lesions in the LLL of the lung, mediastinal lymph nodes, spines, and ribs. (C) Chest CT scan, after three cycles of pembrolizumab monotherapy, showed a slight increase in both the LLL mass and endobronchial lesion. (D and E) Follow-up chest CT and PET scans after five cycles of treatment showed a marked enlargement of the LLL mass, aggravation of the previously identified metastatic lesions, and several newly developed spine metastases. (F) Next-generation sequencing showed that the tumor harbored *STK11/TP53/KRAS* compound mutations.

additional two cycles of treatment, which was well tolerated by the patients without the occurrence of immune-related adverse events. However, the patient's cough was difficult to control despite the use of antitussives, inhaled bronchodilators, and systemic corticosteroids. Follow-up of patients with chest CT and PET scans conducted in January 2020 showed a marked enlargement of both the primary lung tumor and the mediastinal lymph nodes as well as several newly developed bone metastases (Figure 1D and E). The carcinoembryonic antigen level also elevated to 57.2 ng/mL from the baseline level of 21.9 ng/mL. We immediately discontinued the frontline treatment and switched to cytotoxic chemotherapy (pemetrexed and carboplatin). At the same time, we performed next-generation sequencing (NGS) to isolate genetic alterations that might be related to the poor response to pembrolizumab in our patient. NGS revealed three concurrent mutations in the *KRAS* (p.G12V), *TP53* (p.E286K), and *STK11* (p.Y36fs*) genes (Figure 1F). Soon after the first cycle of chemotherapy, the patient developed pneumonia. Serum C-reactive protein and procalcitonin levels elevated to 12.7 mg/dL (normal: < 0.5 mg/dL) and 1.27 ng/mL (normal: < 0.046 ng/mL), respectively. Additionally, chest CT scan showed definite bronchial obstruction at LLL while no ground-glass opacities or airspace consolidations were evident in the right lung. This indicates an association of the pneumonia with bronchial obstruction rather than immunotherapy and subsequent chemotherapy. Despite intensive care with broad-spectrum antibiotics and mechanical ventilation, the pneumonia quickly progressed to sepsis from which the patient succumbed in February 2020.

Discussion

Cancer immunotherapy with ICIs has shown promising outcomes in numerous clinical trials and has provided one of the most important breakthroughs in the management of solid tumors, including lung cancers.¹¹ Antibodies against PD-1 and PD-L1 have become the standard of care for second-line treatment for all comers with NSCLC, and first-line treatment for the patients with high PD-L1 expression. PD-L1 expression has been consistently shown to correlate with an increased efficacy of anti-PD-1/PD-L1 treatments.^{12–14} The US Food and Drug Administration approved the use of anti-PD-L1 clones, 22C3 and 28–8, as a companion diagnostic method for pembrolizumab and a complementary diagnostic method for nivolumab, respectively, in 2015. However, PD-L1

expression alone is not sufficient to predict a patient's response to ICIs.¹⁵ Tumor mutational burden (TMB) and concurrent genetic alterations have emerged as novel predictive biomarkers for immunotherapy with immune checkpoint blockade.¹⁶

STK11 (also known as liver kinase B1, *LKB1*) is a tumor suppressor gene encoding a serine/threonine kinase 11 that directly phosphorylates AMP-activated protein kinase (AMPK), regulating lipid, cholesterol, and glucose metabolism in a variety of tissues.⁸ *STK11*-AMPK signaling negatively regulates the mammalian target of rapamycin pathway which is commonly dysregulated in human malignancies.^{17,18} Somatic mutations in *STK11*, which primarily induces a loss-of-function, are present in approximately 30% of the NSCLC patients with an increased prevalence seen in smokers and patients harboring *KRAS* mutations.^{19,20} *STK11* mutations alone do not appear to predict response to chemotherapy; however, the occurrence of *STK11/KRAS* co-mutations have been associated with reduced survival in NSCLC patients in a variety of treatment settings.^{6–8} *TP53* is a DNA binding transcription factor that regulates DNA repair, metabolism, and apoptosis.²¹ *TP53* mutations alone have been associated with poor response and survival in NSCLC patients treated with chemotherapy or surgical resection,^{22,23} while their prognostic significance in *KRAS*-mutant tumors remains inconclusive.^{6,7,9}

Current evidence suggests that mutations in *STK11* and *TP53* exert opposite effects on clinical outcomes of immunotherapy.^{4,7} *STK11* mutations induce T-cell exhaustion and secretion of immunosuppressive cytokines, resulting in uninfamed tumor microenvironment (TME) with weak PD-L1 expression, and have been linked to adverse clinical outcomes in NSCLC patients treated with immune checkpoint blockade therapy.^{4,7,24} In contrast, tumors harboring *TP53* mutations are characterized by frequent somatic mutations, an inflamed TME, and a favorable response to PD-1/PD-L1 inhibition.^{4,7} *STK11/TP53/KRAS* triple mutations are exceptionally rare; only seven in 1385 NSCLC patients harbored this mutation.¹⁰ Thus, the clinical significance of these compound mutations in immunotherapy has never been discussed. Our case demonstrated de novo resistance to frontline pembrolizumab monotherapy despite strong PD-L1 expression. To the best of our knowledge, this is the first case describing an adverse clinical outcome after immune checkpoint blockade for NSCLC harboring *KRAS/STK11/TP53* co-mutations. A recent study by Bange et al demonstrated that *STK11/*

TP53/KRAS triple mutation in advanced NSCLC conferred a better prognosis after first-line therapy compared with *STK11/KRAS* co-mutations or *STK11* alone.¹⁰ Among the sixty-two enrolled patients in that study, fifty-one (82%) were treated with platinum-doublet chemotherapy, while only five patients (8%) received immunotherapy as a first-line treatment.¹⁰ Therefore, the effect of *STK11/TP53/KRAS* co-mutations on survival was evaluated mainly in patients treated with chemotherapy. In addition, only seven patients with *STK11/TP53/KRAS* co-mutations were enrolled in that study. Those previous results and ours suggest that the survival impact of the compound mutations can be different depending on treatment settings. The mechanism underlying the primary resistance of *STK11/TP53/KRAS*-mutant lung adenocarcinoma to immune checkpoint blockade in our case remains unclear; however, it may be partially explained by the strong proliferative drive and profound immune escape mediated by the mutations in each of these genes as described in previous studies.^{6,7} Interestingly, previous studies have demonstrated that *TP53* has four potential binding sites in the *STK11* promoter region, suggesting that it may act as a regulator of *STK11*.^{25,26} Thus, it may be possible that mutations in *TP53* modulate *STK11* signaling, which may accentuate the uninflamed phenotype of TME. This hypothesis should be validated by future studies.

In summary, we described a case of rare *STK11/TP53/KRAS*-mutant lung adenocarcinoma which demonstrated resistance to anti-PD-1 therapy. Although further evidence should be accumulated, our report suggests that this compound mutation may be a strong predictive biomarker for adverse treatment outcomes in ICIs, and may constitute a distinct aggressive subtype requiring unique therapeutic strategies other than immunotherapy, even when the tumor strongly expresses PD-L1. In addition, our case emphasizes the importance of comprehensive molecular profiling in routine clinical practice, even in this treatment setting, and supports the development of clinical trials to facilitate “personalized immunotherapy” for genetically defined subsets of NSCLC.

Consent for Publication

Written informed consent was obtained from the patient's wife for the publication of both the case report and its accompanying images.

Data Sharing Statement

All data generated or analyzed during this study are available in this manuscript.

Ethics Statement

The publication of this case report was approved by the Institutional Review Board at Kyung Hee University Hospital (KHUH 2020-06-010).

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Disclosure

The authors report no conflicts of interest in this work.

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