



The role of concurrent amplification of PD-L1, PD-L2 and JAK2 in metastatic lung adenocarcinoma as a biomarker of immune checkpoint inhibitor response: a case report

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Background: Immune checkpoint inhibitors (ICIs) have transformed the landscape of care for many malignancies, including non-small cell lung cancer (NSCLC). That said, ICIs are associated with significant immune related adverse events (IrAE) and most patients do not benefit. As a result, significant efforts have been devoted to identifying biomarkers able to predict response to ICIs.

Case Description: This report highlights a patient with diffusely metastatic NSCLC (adenocarcinoma) with RET translocation (RET-KIF5B fusion) who experienced an exceptional response to nivolumab. She was initially treated with standard of care platinum-based chemotherapy with a mixed response and significant toxicity after 3 cycles. She subsequently received 43 cycles of nivolumab on an expanded access program. In total, her disease remained stable 43 months after nivolumab had been stopped. On genetic testing, the patient's tumor was found to harbour amplification of PD-L1, PD-L2 and JAK2.

Discussion: This case highlights the potential value of PD-L1, PD-L2 and JAK2 gene cluster amplification as a biomarker of ICI sensitivity. Importantly, amplification of this gene cluster appears to confer sensitivity to nivolumab regardless of the presence of a RET rearrangement, a known driver mutation in NSCLC. This raises the question whether routine testing for rare but strong markers of ICI sensitivity can improve selection of upfront systemic therapy in patients with NSCLC.

Keywords: Non-small cell lung cancer; anti-PD-1; immune checkpoint inhibitor; case report; PD-L1 amplification

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Introduction

Lung cancer is the second most common cancer and the leading cause of cancer mortality worldwide (1). Approximately 80-85% of lung cancer cases are classified as non-small cell lung cancer (NSCLC) with adenocarcinoma being the most common histology type, representing around 50% of all lung cancer cases (2). Traditionally, the mainstay

of systemic treatment for advanced NSCLC has been platinum-based chemotherapy. In recent years, immune checkpoint inhibitors (ICI) and tyrosine kinase inhibitors (TKIs) have transformed therapy. ICIs, specifically those targeting the PD-1/PD-L1 axis, are now used routinely in patients with advanced NSCLC.

Despite these advances, most patients treated with

* The subject of this report, who is responsible for the Patient Perspective section and agrees to be recognized as one of the authors.

ICIs do not respond. In patients with heightened expression of PD-L1 ($\geq 50\%$ of tumor cells), a marker of favorable ICI response in NSCLC, only 45% responded to Pembrolizumab as first line therapy (3). Likewise, nivolumab demonstrates a 20% response rate in the second line setting (4). These findings, in combination with the risk of developing immune related adverse events (irAE) and the substantial cost of these agents, have fueled the search for biomarkers that predict response to ICIs. In addition to PD-L1 immunohistochemical staining, total mutation burden (TMB), mismatch repair system deficiency (dMMR) and tumor-infiltrating lymphocytes have all been associated with an increased likelihood of benefit from ICIs (5). PD-L1 expression is the only marker used routinely in NSCLC with proven predictive value. That said, many patients with PD-L1 negative tumors experience durable responses, signaling that continued investigation of predictive biomarkers is needed.

PD-L1 amplification is associated with sensitivity to ICIs in NSCLC (6). PD-L1 and PD-L2 are ligands that bind PD-1 on activated T-cells and signal for down regulation of immune activity. Tumor cells can exploit this interaction by over-expressing PD-L1 on their cell surface (7). Amplification of *PD-L1* is rare in solid tumors, having been reported in only 0.7% of cases tested (8). It is commonly linked with co-amplification of *PD-L2* and *JAK2*, as all three genes are coded for in close genomic proximity on chromosome 9p24.1 (9). Currently, testing for *PD-L1* amplification is not performed routinely in clinical practice.

In this report, we present a case of lung adenocarcinoma,

with *RET* translocation who demonstrated a robust response to nivolumab after upfront treatment with 3 cycles of cisplatin and pemetrexed. This patient was found to harbour amplification of *PD-L1*, *PD-L2* and *JAK2* on genetic testing. We present the following case in accordance with the CARE reporting checklist (available at <https://pcm.amegroups.com/article/view/10.21037/pcm-22-39/rc>).

Case presentation

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

A Caucasian 47-year-old female with no past medical history or smoking history presented to her family physician in May 2015 for a prolonged dry cough with hemoptysis and non-pleuritic chest pain starting 4 months ago. No history of occupational exposures to asbestos. Her family history was notable for breast cancer in her mother at age 75. She underwent a chest X-ray that was markedly abnormal and proceeded to have a computed tomography (CT) chest, which showed a large left lower lung cavitary mass with thrombosis within the left inferior pulmonary vein. Bronchoscopy was performed and a biopsy was taken, which showed invasive adenocarcinoma that stained positive for TTF-1. Testing for EGFR and ALK mutations was negative. Her only medication at the time of medical oncology consultation was low-molecular weight heparin (LMWH), which was prescribed for the left inferior pulmonary vein thrombosis. Her Eastern Cooperative Oncology Group (ECOG) performance status was 1. Her staging positron emission tomography (PET) scan showed metastases to the right proximal femur, right adrenal gland, enlarged supraclavicular and paratracheal nodes, as well as multiple mesenteric lesions. Magnetic resonance imaging (MRI) brain revealed a 9 mm lesion in right temporal lobe, see *Figure 1*. She was started on cisplatin/ pemetrexed at standard dosing, in June 2015. She also completed radiation to right femur comprising 20 Gy in 5 fractions.

In July 2015, the patient completed her third cycle of chemotherapy, but experienced significant gastrointestinal toxicities of non-bloody diarrhea, nausea/vomiting and

Highlight box

Key findings

- This is a case of metastatic NSCLC demonstrating an exceptional response to nivolumab.
- Genetic testing revealed amplification of the *PD-L1/PD-L2/JAK2* gene cluster.

What is known and what is new?

- There is still need for better markers of response to immune checkpoint inhibitors.
- Amplification of the *PD-L1/PD-L2/JAK2* gene cluster may be a strong marker of anti-PD-1 therapy response.

What is the implication, and what should change now?

- This case questions whether routine genetic testing for *PD-L1/PD-L2/JAK2* amplification could optimize the choice of upfront systemic therapy in NSCLC.

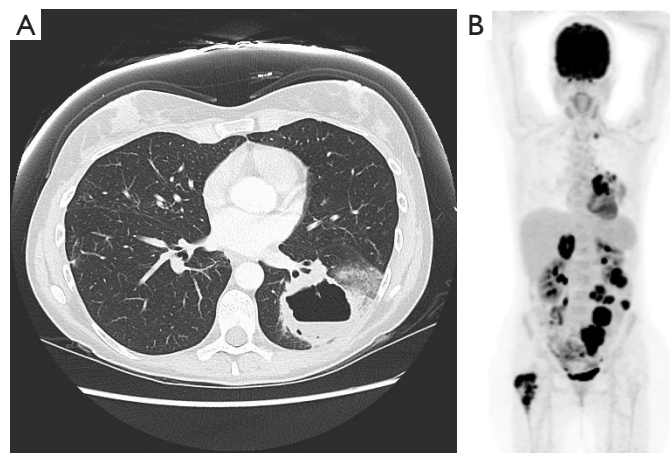


Figure 1 Imaging from original staging work-up at the time of diagnosis. (A) Axial image of the patient's initial CT chest with contrast showing the large left-sided cavitory lesion representing the primary tumor. (B) PET scan from June 2015 was performed as part of the patient's staging work-up. There is significant enhancement in the proximal left femur, right adrenal gland, and multiple mesenteric lesions, in keeping with widespread metastatic disease. CT, computed tomography; PET, positron emission tomography.

abdominal discomfort. Restaging CT scans demonstrated a mixed response: improvement of her supraclavicular/mediastinal adenopathy, no change to the primary tumor's size but it was now fluid-filled and showed invasion of the left pleura. Her abdominal metastases and adrenal metastases enlarged. She started treatment with nivolumab through a compassionate access program.

The patient was admitted to hospital in September 2015 for a perforation of a small bowel metastases requiring exploratory laparotomy and small bowel resection with primary anastomosis. An intra-abdominal abscess was also drained. This resulted in a 5-week delay between her first and second dose of nivolumab. She received genetic testing by Foundation Medicine that revealed a *PD-L1*, *PD-L2* and *JAK2* amplification. The *RET-KIF5B* fusion was also identified. The full results are available in *Table 1*.

The patient continued nivolumab and experienced an excellent response at all disease sites, including her primary site, see *Figure 2*. In February 2016, a single enlarging lymph node was observed. Endobronchial ultrasound (EBUS) biopsy revealed a noncaseating granuloma in keeping with sarcoid reaction.

The patient's experienced two short breaks from nivolumab occurred between January–March 2016 (cycle 11) and May–July 2016 (cycle 18) due to prolonged grade 1–2 diarrhea. Treatment resumed thereafter and the patient remained in good health aside from a partial small bowel obstruction from adhesions released surgically, keratoconjunctivitis sicca managed with artificial tears and oligo-inflammatory

arthritis beginning in the left knee. The latter two symptoms were felt to represent IrAEs. In September 2017 (cycle 41), her diarrhea progressed to grade 3 level toxicity. At that point, her nivolumab was held indefinitely and she began a prednisone taper starting from 45 mg and tapering over 9 weeks. She was followed by rheumatology and prescribed methotrexate as maintenance therapy for her oligoarthritis. Serial restaging imaging was performed every 3–4 months.

In December 2020, after 43 months off treatment, the patient developed isolated mediastinal lymph node enlargement, which was in keeping with recurrent metastatic adenocarcinoma on EBUS biopsy. The patient was restarted on nivolumab March 2021. Her latest CT scans from May 2022 reveal stable mediastinal lymph nodes and multiple small nodular lung lesions with changes in keeping with inflammation rather than malignancy. An X-ray performed in June 2022 showed a new pleural effusion. The patient started a course of steroids for pleuritis, related to ICI therapy and nivolumab has been held. There is no evidence of disease progression at other sites.

Patient perspective

I am grateful for the opportunity to contribute to this case study on my treatment and response as a Stage 4 NSCLC patient. I am also grateful to my medical team for their outstanding support and care. Treatment options for lung

Table 1 The results of Foundation Medicine Genetic Testing in 2015 from a core needle biopsy of the patient's left lower lobe mass

Gene	Type of mutation	Copy number change	FDA approved therapies [#]
<i>CCND1</i>	Amplification	9	Palbociclib
<i>CD274 (PD-L1)</i>	Amplification	8	Nivolumab, pembrolizumab
<i>JAK2</i>	Amplification	8	Ruxolitinib
<i>PDCD1LG2 (PD-L2)</i>	Amplification	8	Nivolumab, pembrolizumab
<i>RET</i>	KIF5B-RET fusion	N/A	Carbozantinib, lenvatinib, ponatinib, regorafenib, sorafenib, sunitinib, vandetanib
<i>CDKN2A/B</i>	Loss	N/A	None
<i>FGF19</i>	Amplification	9	None
<i>FGF4</i>	Amplification	9	None
<i>MYC</i>	Amplification	21	None
<i>RICTOR</i>	Amplification—equivocal	7	None
<i>TP53</i>	R213 [#]	N/A	None
<i>FGF3</i>	Amplification	9	None
<i>NKX2-1</i>	Amplification—equivocal	7	None

[#], FDA approved therapies for any tumor type at the time of genetic testing [2015]. FDA, Federal Drug Agency.

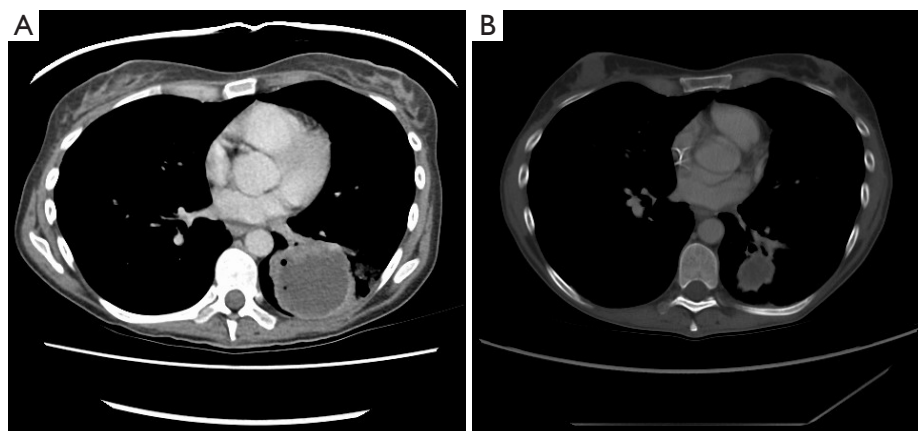


Figure 2 Axial slices of the patient's CT chest. (A) Prior to the start of nivolumab September 2015 showing a large left-sided mass. (B) October 2016, 14 months after the initiation of nivolumab, showing interval decrease in size of the left lung mass. CT, computed tomography.

cancer remain extremely limited although it is the most commonly diagnosed and deadliest form of cancer in North America. My response to nivolumab has been extraordinary, particularly given that we had limited information to guide decision-making as well as limited treatment options in 2015. Yet 7 years later, I am well and have more treatments options available than when I was diagnosed (Figures S1,S2).

Next-generation sequencing has been critical to developing a better understanding of my cancer and its

response to nivolumab, as well as to identifying the *RET-KIF5B* fusion in my tumour cells. I strongly believe that all cancer patients should have access to full panel biomarker testing so that they can access the best available treatments tailored to their specific form of cancer.

Knowledge that I have *PD-L1* amplification and the *RET* fusion alteration have been a source of needed optimism and hope for me and my family as we have lived through this challenging journey. I hope that my

case study will contribute to research that will generate more viable treatment options for lung and other cancer patients. I also hope that it will provide needed optimism and hope for patients and their families as we move closer to finding a cure.

Discussion

The search for biomarkers that predict response to ICIs in NSCLC is an active area of investigation. In this case of metastatic lung adenocarcinoma in a non-smoking patient, we identified amplification of *PD-L1*, *PD-L2* and *JAK2* that was associated with a long-lasting response to nivolumab.

The *PD-L1* gene is coded for on chromosome 9p24.1. This chromosomal segment also encodes *PD-L2* and *JAK2*. Amplification of this gene cluster has been identified in several malignancies, including triple negative breast cancer, diffuse large B-cell lymphoma, Hodgkin's lymphoma, and urothelial cancer (10-12). The presence of *PD-L1* amplification is associated with poorer prognoses in certain cancers, but also an increased sensitivity to ICIs (9). This was demonstrated in a recent trial of Hodgkin's lymphoma patients failing first-line therapy with chromosome 9.24.1 amplification that showed an 86% overall response rate with nivolumab (13). It is proposed that *PD-L1* amplified tumors display heightened immunotherapy sensitivity through upregulation of PD-L1 expression on tumor cell surfaces. In the case of chromosome 9p24.1 amplification, elevated JAK2 expression may be synergistic in upregulating PD-L1 expression through phosphorylation of STAT3 and STAT5, resulting in enhanced *PD-L1* promoter activity (14). That said, the fact that *PD-L1* amplification does not always correlate with PD-L1 expression raises the question whether there is a more complex interaction between PD-L1/PD-L2/JAK2 that confers sensitivity to ICIs irrespective of *PD-L1* gene expression (15). Further work is needed to clarify this.

PD-L1 amplification is a rare finding in NSCLC with an estimated prevalence of 3%. When identified, it is usually associated with squamous histology and a history of smoking. In patients with adenocarcinoma, *PD-L1* amplification is only identified in 0.6% of patients (8). Despite the low prevalence of *PD-L1* amplification, it is a strong marker of ICI response when present. In a series of 5 patients collected prospectively with NSCLC and *PD-L1* amplification, Inoue *et al.* found an 80% overall response rate with nivolumab (15). One limiting factor was the median follow-up duration of 12.6 months. Our

case is a unique addition to this study, as it demonstrates a prolonged response to ICIs in a patient with a high burden of metastatic disease and greater than 7 years of follow-up.

RET driver mutations are found in approximately 1–2% of NSCLC (16). The *RET-KIF5B* fusion results in the formation of a supercoiled KIF5B domain that can homodimerize leading to activation of RET kinase and downstream signaling to the Ras-MAPK and PI3K-Akt pathways (17). These mutations are targetable by RET kinase inhibitors, such as Selpercatinib (18). Like most NSCLC containing a driver mutation, the presence of a *RET* driver mutation is associated negatively with response to ICIs (19). Interestingly, this patient was never treated with a RET kinase inhibitor and had an excellent response to nivolumab. This raises the question whether amplification of chromosome 9p24.1 predicts patients who will benefit from ICIs even in the setting of a known driver mutation. This would make for an appealing biomarker, as ICIs tend to lead to more long-lasting and robust responses.

Another important consideration is the relationship between IrAEs and response to ICI therapy. Patients who experience IrAEs have a tendency for improved outcomes on treatment (20). In this case, the patient experienced grade 1–2 colitis, oligoarthritis and keratoconjunctivitis. The link between *PD-L1/PD-L2/JAK2* co-amplification and adverse events represents an area of future investigation.

This report is limited in that it reports the experience from a single patient, therefore conclusions of causation are not possible. Furthermore, this patient was initially treated in 2015 when quantification of PD-L1 expression was not routinely performed. This patient already showed exceptional response to nivolumab, so testing was deferred as it was not thought to change management. There were limitations to the Foundation Medicine Assay, as fluorescence in situ hybridization (FISH) is not included in its package. As a result, genetic testing was unable to confirm whether *JAK2*, *PD-L1* and *PD-L2* amplification arose from a single amplification of the genetic region. Given the close genomic proximity of *PD-L1/PD-L2/JAK2* and equivalent gene copy numbers, we felt that amplification of the entire chromosomal locus was more likely than independent mutations.

Conclusions

In conclusion, this is a case of NSCLC with amplification of *PD-L1/PD-L2/JAK2* exhibiting an excellent and long-lasting response to ICIs. Although amplification of this

gene cluster is rare in NSCLC adenocarcinoma, it is a meaningful and actionable finding. This raises the question whether routine genetic testing for *PD-L1/PD-L2/JAK2* amplification could optimize the choice of upfront systemic therapy and ultimately improve outcomes in this patient cohort.

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Footnote

Reporting Checklist: The authors have completed the CARE reporting checklist. Available at <https://pcm.amegroups.com/article/view/10.21037/pcm-22-39/rc>

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://pcm.amegroups.com/article/view/10.21037/pcm-22-39/coif>). BL has served on advisory boards for AstraZeneca, Pfizer, Bayer, Novartis and Roche. The molecular oncology diagnostics laboratory, for which he serves as Medical Director, has received research and quality improvement grant supports from Amgen, AstraZeneca, Roche and EMD Serono. The above advisory boards and work supported by the grants have not dealt directly with specific issues raised in the manuscript. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office of this journal.

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Figure S1 Patient's family photo pre-diagnosis in 2015. This image is published with the patient's consent.



Figure S2 Patient's family photo 6 years after diagnosis in 2021. This image is published with the patient's consent.