Early Application of Next-Generation Sequencing Identifies Pancreatic Mass as Metastasis From an EGFR-Mutated Lung Adenocarcinoma

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ABSTRACT

Pancreatic metastasis of primary lung adenocarcinoma is a rare occurrence, accounting for <0.3% of all pancreatic malignancies. Given that the prognosis and treatment options for primary pancreatic cancer differ greatly from pancreatic metastases from a primary site, an accurate diagnosis is critical. This report presents a unique case of a 65-year-old man who was admitted with significant unintentional weight loss, fatigue, abdominal pain, and jaundice, and found to have a pancreatic mass initially thought to be primary pancreatic adenocarcinoma and subsequently diagnosed as an EGFR-mutated lung adenocarcinoma with metastases to the pancreas via early application of next-generation sequencing (NGS). The use of NGS early in the patient’s clinical course not only changed the treatment strategy but also drastically altered the prognosis. Although metastatic pancreatic adenocarcinoma has a poor prognosis and survival rate, treatment of EGFR-mutated non–small cell lung cancer with EGFR tyrosine kinase inhibitors is associated with high response rates. Importantly, our case demonstrates that timely application of NGS very early in the disease course is paramount to the diagnosis, management, and prognosis of solid malignancies.

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Case Report

A 65-year-old White man with no smoking history and a past medical history of hypertension, hyperlipidemia, hypothyroidism, asthma, obstructive sleep apnea, and depression presented to the emergency department with worsening abdominal pain. He experienced a significant unintentional 35-pound weight loss over 6 months with associated fatigue, abdominal pain, and light-colored stools. Physical examination findings were notable for scleral icterus and jaundice. In the emergency department, his liver function test results showed elevated values, with a total bilirubin level of 17.1 mg/dL, alkaline phosphatase level of 1,138 U/L, aspartate transaminase level of 332 U/L, and alanine transaminase level of 846 U/L. A CT scan of the abdomen and pelvis revealed a mass measuring approximately 25 × 16 mm in the uncinate process of the pancreas extending to the pancreatic head and body with intrahepatic and extrahepatic biliary ductal dilation (Figure 1A) and low-density changes through the pancreatic body (Figure 1B).

The patient underwent an endoscopic retrograde cholangiopancreatography (ERCP) with sphincterotomy and placement of a metal stent to the distal common bile duct stricture. Endoscopic ultrasound-guided core biopsies of the pancreatic head and body revealed a diagnosis of adenocarcinoma. No peripancreatic lymph nodes were seen. At 2 weeks following stent placement, the patient’s CA 19-9 level was 97 U/mL. At the time of initial diagnostic biopsy, next-generation sequencing (NGS) was obtained via Tempus Laboratories, Inc. Staging CT scan of the chest showed suspicious spiculated nodules within the right upper lobe posterior segment (measuring 12 × 8 mm) of the lung (Figure 2) and central left upper lobe (not shown), which were inconclusive. After conferring with a multidisciplinary team, the patient’s pancreatic tumor was determined to be technically resectable but would require neoadjuvant chemotherapy prior to surgery given the large tumor burden and diffuse nature.
of the mass within the pancreas, as well as the suspicious lung nodules. Biopsy of the right lung nodule was planned.

Prior to initiation of modified FOLFIRINOX (mFFX; folinic acid/5-FU/irinotecan/oxaliplatin) as neoadjuvant therapy, NGS of the pancreatic tissue revealed EGFR (OMIM #131550) S768I and G719C gain of function (GoF) mutations. The S768I and G719C mutations are located in exons 20 and 18, respectively, within the tyrosine kinase domain of the EGFR protein.1 A complete list of biologically relevant genomic mutations is provided in Table 1.

Subsequent immunostaining of the pancreatic tissue biopsy showed reactivity for thyroid transcription factor-1 (TTF-1) and napsin A (Figure 3). These results raised suspicion that the patient’s tumor may not be a primary pancreatic malignancy. TTF-1 and napsin A have been shown to be useful diagnostic markers in distinguishing primary lung adenocarcinoma from metastatic carcinoma in the lung,2,3 whereas EGFR is a frequently altered gene in non–small cell lung adenocarcinomas.4 The findings suggested that the lung, rather than the pancreas, was likely the primary site of the patient’s tumor origin. mFFX treatment was not administered. Subsequently, core biopsies of the spiculated pulmonary nodule seen on the staging CT scan showed similar histomorphology, immunoprofiling, and genetic mutations as the previous pancreatic biopsy, confirming primary non–small cell adenocarcinoma of the lung with pancreatic metastasis.

The patient initiated osimertinib therapy. At a 3-month follow-up visit, he reported feeling better overall, with improved appetite and a weight gain of 15 pounds. Follow-up CT scan of the chest and abdomen showed improvement of the right upper lobe nodule and decrease of the pancreatic head mass (Figure 4).

Informed consent was obtained from the patient to publish this case report.

Discussion

Metastasis to the pancreas from a solid primary malignancy is a rare occurrence, with approximately 2% of pancreatic masses originating from a different primary site, most commonly from renal, breast, melanoma, lung, and colorectal primaries.5 Pancreatic metastases from lung primaries account for approximately 9% to 14.7% of all pancreatic metastases, representing <0.3% of all pancreatic malignancies.6,7 Pancreatic secondaries are rarely diagnosed simultaneously with the primary tumor because they are often asymptomatic. The reported mean interval between primary diagnosis of lung carcinoma and identification of pancreatic metastasis is 2.4 years,6 with many pancreatic secondaries being discovered on routine follow-up scans. In our patient case scenario, nodules in the right upper lobe and left upper lobe were noted during staging after the initial diagnosis of pancreatic cancer. Given that lung
metastases from pancreatic cancer are reported to be found in approximately 20% of patients, together with the histologically confirmed diagnosis of pancreatic adenocarcinoma, the discovery of suspicious lung nodules on the staging CT scan was thought to be potentially metastasis from a primary pancreatic malignancy, and the standard treatment course for both neoadjuvant and frontline metastatic pancreatic ductal adenocarcinoma (PDAC)—mFFX—was initially planned as first-line treatment for the patient.

Current NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) for Pancreatic Adenocarcinoma recommend genetic testing for inherited mutations, microsatellite instability testing, and/or mismatch repair deficiency testing in all patients with histologically confirmed pancreatic cancer. Tumor/somatic molecular profiling is only recommended for patients with locally advanced or metastatic disease, with no specifics given on timing. In this case, although the patient’s tumor was technically classified as resectable, with suspicious but nonspecific nodules in the lung noted, somatic testing was immediately ordered at the time of diagnosis and revealed EGFR S768I and G719C GoF mutations in exons 20 and 18, respectively. EGFR is known to be highly conserved in pancreatic cancers but is commonly mutated in non–small cell lung cancer (NSCLC), indicating that the initial working diagnosis of pancreatic adenocarcinoma was unlikely. Thus, further workup of the lung nodule was completed prior to initiation of mFFX neoadjuvant therapy, and treatment for EGFR-mutated NSCLC with osimertinib was initiated instead.

Use of NGS very early in the patient’s diagnostic workup not only changed the treatment paradigm but also drastically altered the primary tumor diagnosis and the patient’s prognosis, given that EGFR-mutated NSCLC has a response rate of approximately 70%. NGS is a highly sensitive tool that allows for the quantitative analysis and simultaneous evaluation of multiple genes that, when mutated or altered, can serve as actionable biomarkers for targeted therapy. The number of oncogenic driver alterations and targeted therapies to treat the alterations have increased in NSCLC in the past 2 decades. Mutations in the EGFR gene are some of the most common targetable driver mutations found in NSCLC. Other genotypes that can be identified by NGS and have potential targeted therapy available include ALK, ROS1, NTRK, and RET rearrangements; MET abnormalities; and KRAS, BRAF, and HER2 mutations.

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**Table 1. Mutation Profile of Pancreatic Biopsy**

<table>
<thead>
<tr>
<th>Biologically Relevant Genomic Variants</th>
<th>Variant Type</th>
<th>Variant Allele Fraction</th>
</tr>
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<tbody>
<tr>
<td>EGFR S768I</td>
<td>Missense (exon 20) GoF</td>
<td>57.30%</td>
</tr>
<tr>
<td>EGFR G719C</td>
<td>Missense (exon 18) GoF</td>
<td>53.90%</td>
</tr>
<tr>
<td>CTNNB1 S37F</td>
<td>Missense GoF</td>
<td>31.80%</td>
</tr>
<tr>
<td>SMAD4 W323fs</td>
<td>Frameshift LoF</td>
<td>30.40%</td>
</tr>
<tr>
<td>RASA1 Q546fs</td>
<td>Frameshift LoF</td>
<td>14.60%</td>
</tr>
<tr>
<td>SMAD4 Q366</td>
<td>Stop gain LoF</td>
<td>10.90%</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>Copy number loss</td>
<td>N/A</td>
</tr>
<tr>
<td>CDKN2B</td>
<td>Copy number loss</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Abbreviations: GoF, gain of function; LoF, loss of function; N/A, not available.

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**Figure 3.** Immunohistopathologic analysis of fine-needle core biopsy of the pancreas confirming adenocarcinoma. (A) Hematoxylin-eosin staining of the tumor. (B) Positive immunostaining for thyroid transcription factor-1. (C) Positive immunostaining for napsin A (original magnification, ×20 for all).
Mutations in \( EGFR \) tyrosine kinase occur in approximately 15% of NSCLC adenocarcinomas in the United States, with a higher incidence in nonsmokers and the Asian population.\(^{13,14}\) \( EGFR \) is a member of the transmembrane receptor family. Upon activation via ligand binding, \( EGFR \) undergoes autodimerization and heterodimerization in a proliferative signaling pathway that enhances cellular multiplication, attenuates apoptosis, and triggers expression of angiogenic growth factors.\(^{15–17}\)

Two of the most common \( EGFR \) mutations are deletions in exon 19 (del19) and a single amino acid substitution in exon 21 (L858R), which together account for approximately 85% of all \( EGFR \) mutations in NSCLC. The remaining “rare” mutations include point mutations, insertions, and deletions in exons 18–25 of the \( EGFR \) gene.\(^{18,19}\) The presence of an \( EGFR \) mutation in advanced NSCLC results in a more favorable prognosis and predicts sensitivity to treatment with \( EGFR \) tyrosine kinase inhibitors (TKIs). Thus, targeted therapy with \( EGFR \) TKIs is the preferred frontline treatment of \( EGFR \)-positive NSCLC and should be considered prior to chemotherapy and immunotherapy.\(^{20}\) Data demonstrate the superiority of osimertinib over standard \( EGFR \) TKIs as first-line treatment for advanced \( EGFR \)-mutant NSCLC in terms of both progression-free and overall survival.\(^{21,22}\)

Osimertinib is a third-generation \( EGFR \) TKI that irreversibly binds via the C797 amino acid covalent bond to mutant forms of \( EGFR \), inhibiting DNA synthesis and proliferation through various downstream pathways such as AKT, MAPK, and RAS.\(^{23}\) For uncommon mutations such as G619C and S768I, osimertinib has also demonstrated activity.\(^{24}\) After 3 months of therapy, our patient showed significant clinical improvement while tolerating osimertinib therapy well, due to the very early implementation of NGS somatic testing for presumed PDAC.

In addition to NGS, early application of histopathologic immune staining, including TTF-1 and napsin A, provided valuable information in aiding the diagnosis of a lung primary adenocarcinoma prior to initiation of therapy. TTF-1 is a master regulator of transcription and plays an important role in maintaining terminal respiratory unit cell function in the normal lung. It is commonly used to differentiate primary pulmonary adenocarcinoma from secondary metastasis to the lung.\(^{2}\) Another pulmonary-specific marker is napsin A, an aspartic proteinase that is involved in the maturation of surfactant proteins and has been shown to be more sensitive and specific than TTF-1 in distinguishing primary lung adenocarcinoma from metastatic lung adenocarcinoma.\(^{25}\) Thus, early histopathologic and immunohistochemical analysis should be taken into consideration upon histologic confirmation of adenocarcinoma for suspected primary tumor sites.

NGS use remains limited in early-stage pancreatic cancers; however, increasing use of both somatic and germline testing could reveal additional treatment information and guide research for pancreatic cancer. Most often, somatic testing in PDAC is obtained in the advanced setting following surgical resection or biopsy of metastatic lesions. Somatic and germline mutations in tumor tissue are classically identified using the “tumor-
normal” sequencing design, in which cancerous tissue is compared with a matched sample of noncancerous tissue to identify which mutations are unique to the tumor.\textsuperscript{26} When matched samples are not available, tumor mutations can be compared with a database such as gnomAD (Genome Aggregation Database), a large and publicly available consortium of genome and exome sequencing data that reflects population variation on an unprecedented scale.\textsuperscript{27} One study of 336 patients with PDAC reported that NGS identified potentially actionable findings in 26% of the cohort.\textsuperscript{28} This highlights the need for early implementation of sequencing at the time of diagnosis to identify subsets of patients with pancreatic cancer who may have mutations that can be treated with targeted oncologic therapies or for more appropriate trial selection throughout the disease course. Moreover, in patients with potentially resectable disease, those who are also high risk (eg, large tumor burden, pain, or elevated tumor markers [CA 19-9]), or when there is suspicion of metastasis in equivocal lesions, NGS testing should be considered up-front. Contemporary research has identified the primary driving mutations for PDAC, including \textit{KRAS}, \textit{TP53}, \textit{SMAD4}, and \textit{CDK2NA}. Nonetheless, germline and somatic testing of these tumors early in the disease course is still vitally important, not only as demonstrated in this case but also as novel treatments are developed and tested for potentially resectable and advanced PDAC. Additionally, there is emerging evidence of benefit with platinum-based chemotherapy and PARP inhibition for patients with homologous recombination deficiency or mutations in somatic \textit{BRCA1} and germline \textit{BRCA2} genes.\textsuperscript{29,30} Variant allele frequency, which is available in NGS platforms, can also provide insight into somatic and germline mutational status, which can consequently have implications for therapy selection in the neoadjuvant and more advanced settings. Contemporary research is investigating utilizing NGS data to determine novel predictive and prognostic markers or gene signatures that can guide clinical treatment planning.\textsuperscript{31}

**Conclusions**

In our patient’s case, timely use of NGS revealed the presence of an \textit{EGFR} mutation in which targeted therapy, an EGFR TKI, has been shown to improve progression-free and overall survival for advanced \textit{EGFR}-mutant NSCLC. Timely use of NGS was informative in this case, leading to significant treatment and prognostic implications. Increased somatic and germline testing early in the diagnostic process offers the best potential to identify appropriate treatment planning, as well as enables exploration of new treatment avenues, predictive markers, and approaches to clinical trial selection for patients with advanced and aggressive malignancies such as lung and pancreatic cancers.

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