Detection of concurrent hematologic malignancies in solid tumor NGS testing may cause false-positive results

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Next-generation sequencing is becoming the standard of care in the diagnostic workup of lung adenocarcinoma and other solid tumors. This technology leverages massively parallel sequencing to interrogate multiple genes of interest in a single test. The results of NGS have important implications for patient care, providing diagnostic, prognostic, and predictive information.

False-positive NGS results may arise due to multiple scenarios, for example, misidentification of a germline finding as a somatic finding. These false-positive results may lead to misinterpretation or inappropriate use of the NGS results and have serious clinical impact. Here, we discuss a case of NGS solid tumor testing revealing mutations from the patient’s concurrent hematologic malignancy.

Case. An 82-year-old female with chronic myelomonocytic leukemia (CMML) and a remote history of tobacco smoking presented to the emergency department with dyspnea and cough for several weeks that had failed to improve with antibiotics. A chest x-ray and subsequent CT scan without contrast were performed, revealing a 2-cm right upper lobe spiculated lesion. Lung adenocarcinoma was diagnosed on fine-needle aspiration of the nodule, leading to a lobectomy resection of the patient’s right upper lobe. Histologic evaluation of the resection specimen revealed a poorly differentiated pleomorphic carcinoma consisting of adenocarcinoma and giant cell carcinoma with visceral pleura involvement and lymphatic invasion (Fig. 1).

Targeted next-generation sequencing was performed following microdissection of formalin-fixed, paraffin-embedded tissue from the FNA cell block and resection specimens using the ion semiconductor-based sequencing platform Ion Torrent Personal Genome Machine (Thermo Fisher Scientific) with the Ion AmpliSeq Cancer Hotspot Panel v2 (Thermo Fisher Scientific). The panel concurrently interrogates 2,800 hotspots/variants with 207 amplicons in 50 cancer-related genes. Sequence data analysis and variant calling were performed with Torrent Suite Software 5.0 (Thermo Fisher Scientific).

The cytology specimen had a visually estimated tumor cellularity (or neoplastic content) of 25 percent, while the surgical pathology resection specimen had a higher tumor cellularity of 70 percent. Sequencing of the lung cancer specimens revealed multiple concurrent mutations as follows (Table 1, page 2):
- c.34G>T missense mutation (NM_004985, p.Gly12Cys) in the...

Fig. 1. A. Fine-needle aspiration cell block of the lung nodule. B. Low-power view of the lung nodule resection specimen. Inset shows numerous leukocytes packing intratumoral blood vessels on high-power view.
KRAS gene with 22 percent variant allele frequency (VAF) in the FNA and 38 percent in the resection;  
- c.35G>A missense mutation (NM_004985, p.Gly12Asp) in the KRAS gene with three percent VAF in the FNA and six percent in the resection;  
- c.35G>A missense mutation (NM_002524, p.Gly12Asp) in the NRAS gene with 15 percent VAF in the FNA and three percent in the resection; and  
- c.52delA frameshift deletion (NM_000546, p.Thr18His*26) in the TP53 gene with 23 percent VAF in the FNA and 41 percent in the resection.

Due to the finding of multiple concurrent mutations in cancer driver genes (KRAS and NRAS) on NGS testing of the lung adenocarcinoma, the patient’s clinical history regarding her CMML was investigated further. The patient had a reported history of long-standing mild monocytosis since 2009 and elevated hemoglobin at 17 years old, a follow-up complete blood count revealed leukocytosis (14.2 × 10^3/µL [ref: 3.4–11.2 × 10^3/µL]) one week prior to the FNA and 2.6 × 10^3/µL the day of the right upper lobectomy.

We speculated that additional mutations may have been present on the peripheral blood sample for the CMML workup but not reported in the official Genoptix report. The Genoptix pathologist responsible for interpreting the patient’s case was contacted to determine if variants were identified in the sample that were not reported. It was verbally confirmed that an additional mutation was present, a c.35G>A missense mutation (NM_004985, p.Gly12Asp) in the KRAS gene with a VAF of three percent (below their laboratory minimum quality control metric of VAF for reporting, five percent). The TP53 gene is included on the Genoptix myeloid molecular profile panel; however, no TP53 variants were identified in the patient’s sample. Based on the findings in the patient’s next-generation sequencing results from the lung adenocarcinoma, the patient was not eligible for targeted tyrosine kinase inhibitor therapy and received standard chemotherapy with carboplatin and pemetrexed.

**Discussion.** NRAS and KRAS are members of the RAS family of oncogenes. Activating point mutations in these genes have been reported in a variety of tumors, including non-small cell lung cancer and hematological malignancies, mostly concen-

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<th>Specimen</th>
<th>Fine-needle aspiration</th>
<th>Lobectomy</th>
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<tr>
<td>Genetic mutations (VAF %)</td>
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<tr>
<td>KRAS G12C (22%)</td>
<td>KRAS G12C (38%)</td>
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<td>TP53 T18Hfs*26 (23%)</td>
<td>TP53 T18Hfs*26 (41%)</td>
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<td>NRAS G12D (15%)</td>
<td>NRAS G12D (3%)</td>
<td>NRAS G12D (34%)</td>
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<tr>
<td>KRAS G12D (3%)</td>
<td>KRAS G12D (6%)</td>
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<td>No data</td>
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<td>ASXL1 Q760* (43%)</td>
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and are less likely to be in codon 61. NRAS mutations are more commonly identified in lung adenocarcinoma in current and former smokers. KRAS mutations also define a distinct molecular subset of lung adenocarcinoma. While KRAS mutations are found in former and current smokers and never smokers, they are rarer in never smokers and are also less common in patients of East Asian descent. Importantly, KRAS mutations have been reported as an indicator of resistance and poor survival in patients with non-small cell lung carcinoma treated with EGFR-tyrosine kinase inhibitors. The prognostic as well as predictive role of KRAS mutations continues to be studied in lung adenocarcinoma. Although various attempts at inhibiting KRAS have been made, there is no established therapy specific for this large subpopulation of lung cancer patients.

Genetic mutations are common in CMML and are seen in greater than 90 percent of cases. RAS mutations are highly prevalent and are seen in 20–30 percent of CMML, with NRAS mutations more common than KRAS. RAS mutations in CMML have been associated with features of cell proliferation and monocytosis and with shorter survival, although some multivariate models have not substantiated that RAS mutations confer inferior outcome. RAS variants in CMML are often associated with a myeloproliferative phenotype rather than a myelodysplastic phenotype.

Interestingly, it is thought that the initial driver mutation in CMML is likely to be a mutation in TET or ASXL1 and then subsequent secondary mutations, including RAS mutations, may allow clonal subsets to progress. The patient in the case presented here had a nonsense mutation in ASXL1 identified at a notably higher VAF than the RAS variants identified in the same specimen, which probably indicates that the RAS-mutated clones were subpopulations of the larger ASXL1 CMML population. Genetic heterogeneity due to different clonal subpopulations is a well-recognized phenomenon. In most cases of CMML, clonal architecture is mostly linear, but split architecture with several branches arising from the same ancestor have been observed.

Despite being found on NGS testing of the lung FNA and resection specimens, the likelihood of two KRAS variants and an NRAS variant occurring as concurrent somatic alterations in the lung adenocarcinoma is exceedingly low. Most likely, the NRAS G12D and KRAS G12D are somatic mutations in the patient’s CMML, while the KRAS G12C and TP53 variants are truly from the lung adenocarcinoma. The NRAS G12D was found at a higher VAF in the peripheral blood testing (Genoptix) than the solid tumor testing, and NRAS mutations are more common in CMML than lung adenocarcinoma. Additionally, the NRAS G12D has a higher VAF in the FNA cell block than the lobectomy resection specimen. This correlates with the observation that the FNA had significantly lower adenocarcinoma tumor cellularity (25 percent versus 70 percent), with contaminating peripheral blood (along with the patient’s CMML) constituting much of the non-adenocarcinoma cells. While the VAF of the KRAS G12D was slightly higher in the lung resection specimen than the verbally reported VAF for the same mutation in the peripheral blood, this result can be explained by proliferation of the KRAS-mutated CMML subclone between the time of the peripheral blood analysis and the lung cancer resection. The NRAS G12D and KRAS G12D therefore are thought to most likely represent false-positives in the solid tumor NGS testing. These findings could have been further confirmed with analysis of ASXL1 in the solid tumor specimens; however, this gene is not included in the 50-gene panel used in this case.

In this case, the multiple RAS mutations were a sign to further investigate the patient’s clinical history, but this finding may not always be present. In cases in which an expected variant is identified in a particular tumor type—for example, a single KRAS mutation in a lung adenocarcinoma—a false-positive result would not be as obvious. False-positives may have important clinical significance; therefore, inconsistent or unlikely results on NGS testing always warrant investigation of the patient’s history and prior molecular results. Ordering clinicians and pathologists can mitigate the possibility of false-positives owing to secondary malignancies on NGS testing by alerting molecular pathology laboratories to patients’ hematologic diagnoses and prior molecular testing, when applicable.

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