

Case study: pleural fluid cell block with limited tumor content and NGS challenges



Context

B.B., a 62-year-old piano teacher and avid hiker, began experiencing symptoms associated with lung cancer, including sudden shortness of breath and a persistent cough. Concerned, she sought medical help from a pulmonologist referred by her primary care physician. She was found to have a large pleural effusion, and her pulmonologist aspirated 40 mL of fluid, which was sent to pathology. A cell block was prepared, and histology and immunohistochemistry analyses of the cell block confirmed non-small cell lung cancer (NSCLC) with 15% tumor content, making it adequate for molecular testing.

Familiar with the benefits of targeted therapy, her pulmonologist ordered genomic testing with a large next-generation sequencing (NGS) panel to increase the likelihood of identifying an actionable variant. However, after 12 days, the NGS testing was reported as failed due to quantity not sufficient (QNS).

Additional Testing

A liquid biopsy (ctDNA) test was then ordered, returning results after 7 days and detecting a *KRAS* G12S DNA variant at a low variant allele frequency (VAF) of 0.1%. This G12S variant was reported because the performing laboratory felt they had sufficient reads to confidently call it, even though the assay's limit of detection (LoD) was 0.25%.

Seeking confirmatory testing, the medical oncologist referred the pleural fluid cell block to Biofidelity, recognizing the lab's expertise in handling small and challenging sample types.

The sample was sent using four slides, each cut at 5 microns. Both DNA and RNA passed quality control at Biofidelity's laboratory.

Results

Biofidelity's CLIA-certified lab received the sample at 10 a.m. on Wednesday and finalized the valid report by 2 p.m. on Thursday—a turnaround time of just 28 hours. The results revealed a *KRAS* G12V mutation, with no other alterations detected, including the previously reported G12S mutation from the ctDNA assay.

Resolution

Because Biofidelity's laboratory team did not detect a *KRAS* G12S variant but did detect a G12V mutation, Biofidelity reached out to the commercial vendor that initially reported the liquid biopsy result. After further inspection, the vendor confirmed the presence of *KRAS* G12V, just below the threshold for reporting (typically 0.25% VAF). The vendor does not perform germline DNA sequencing to filter out clonal hematopoiesis of indeterminate potential (CHIP) variants, suggesting that the original G12S result was likely a CHIP variant rather than a tumor-related mutation.

Conclusion

This case illustrates the significant advantages of using Biofidelity's Aspyre Clinical Test for Lung in managing NSCLC. If the Aspyre Clinical Test for Lung had been utilized from the start for patient B.B., a confident treatment decision could have been implemented much sooner.

The *KRAS* G12V mutation, present in approximately 3% of NSCLC cases, is associated with an aggressive phenotype, often marked by early recurrence and worse prognosis in some patients. The Aspyre Clinical Test for Lung's ability to detect mutations in samples with low tumor cellularity and challenging quality underscores its value in clinical settings where sample limitations pose significant challenges. Biofidelity's ASPYRE technology not only overcame these barriers due to its high sensitivity and specificity but also demonstrated the capability to detect mutations in an environment with significant noise. The Aspyre Clinical Test for Lung may enhance patient outcomes by improving quality of life through faster and more accurate diagnostic testing.