Cases focus on *ALK* false-negs, post-transplant tumor

William Check, PhD

January 2016—A sophisticated understanding of the advantages and drawbacks of both familiar and advanced assays can provide great patient benefit, as two talks in a session on solid tumor case studies at the Nov. 5–7, 2015 meeting of the Association for Molecular Pathology showed.

Damon Olson, MD, related a "Tale of Two *ALK* False-Negative Lung Adenocarcinomas." One was falsely negative by FISH and the other by immunohistochemistry, both FDA-approved methods.

Dr. Olson

Two methods are approved for screening for *ALK* gene rearrangement in cases of non-small cell lung cancer: a breakapart FISH probe (Abbott Molecular) and, more recently, IHC with the D5F3 antibody (Ventana). In the first patient described by Dr. Olson, who is a fourth-year pathology resident at the University of Colorado, Denver, a lung adenocarcinoma was diagnosed in 2010 and treated. The patient relapsed in 2015. In the initial sample the patient was *ALK* FISH negative, whereas the sample from a 2015 biopsy was *ALK* FISH positive. Retrospective review of the original resection specimen showed that a section from one block from the original resection was FISH negative while a section from a different block from the same resection was positive. IHC results on the two separate blocks showed heterogeneous positivity, suggesting this false-negative result was due to tumor heterogeneity.

A second patient was diagnosed with adenocarcinoma in 2008. Her tumor was FISH positive for *ALK* rearrangement at that time and at subsequent biopsies. These specimens were selected for the laboratory's validation of the D5F3 IHC assay. One of the specimens was negative on the IHC assay, despite being positive by FISH and the tumor showing response to targeted therapy. However, when a freshly cut slide was tested by IHC, it was positive.

Dr. Olson's conclusion: "Beware false results in both modalities which may impact clinical decision-making." He suggested that "High suspicion for ALK rearrangement may need dual testing or additional modalities."

In an interview with CAP TODAY, Dara L. Aisner, MD, PhD, who supervised this work, said that having one false-negative sample for each method highlighted the limitations of both modalities. "It emphasizes my mindset that there is no such thing as a perfect test," says Dr. Aisner, who is co-director of the Colorado molecular correlates laboratory and assistant professor in the university's Department of Pathology. "We have seen other false-negative cases. We picked the most compelling."

Dr. Aisner

She says they have also seen FISH cases that did not have the classic signal pattern required for a positive result. “When we tested the sample by another assay it came out positive. That’s another limitation of the FDA-approved FISH assay—if it’s not clearly positive, it’s negative. There is no room for shades of gray. And we have seen gray in that assay. In my limited experience with the antibody test, it also has shades of gray,” she says.

Dr. Aisner emphasizes that the description of the slide in the second case as “freshly cut” was literal. “If you look carefully, the guidelines for the immuno tell you not to use slides over three months old,” she says. The section that was negative was only 24 days old but still presented a problem. “When we cut the section and put it onto the antibody the same day it was fine,” she says.

As for the laboratory’s current practice, Dr. Aisner says her laboratory’s validation of the IHC test has been completed. “We expect that our standard practice will be to use the antibody test as a first-round screening test with FISH confirmation of all positives and FISH evaluation of all IHC-equivocal samples.” Dr. Aisner also expects to consult with clinicians on any IHC-negative samples that are clinically highly suspicious for rearrangement and to allow access to the FISH assay based on clinician request, regardless of the IHC results.

In the second case, presented by resident Suzanne R. Thibodeaux, MD, PhD, of the Hospital of the University of Pennsylvania, identity testing by PCR was used to determine the origin of a post-transplant tumor. A person who had received a liver transplant nine months previously presented with abdominal pain, diarrhea, and signs of acute kidney injury. Histology and IHC were used to diagnose Kaposi’s sarcoma, which has been associated in rare instances with immunocompromised hosts due to reactivation of human herpes virus 8 (HHV-8).

Pathology was asked to help determine tumor origin—donor or recipient. To do this, the molecular pathology laboratory used PCR to determine the patterns of short tandem repeats in the donor and recipient, using donor gallbladder from the time of the transplant and native recipient liver as identified samples from donor and recipient respectively.

Tumor tissue was circled and macrodissected to get as high a percentage of tumor cells in the extracted DNA sample as possible. Dr. Thibodeaux estimated this sample was 50 percent tumor and 25 percent each stroma and inflammatory cells. Each tissue type could have come from either donor or recipient, giving four combinations. Each combination yielded a distinct percentage of donor and recipient DNA in the mixed sample (possible donor/recipient percent: 0/100, 25/75, 75/25, 50/50).

Data from the short tandem repeat analysis suggest that the tumor was of donor origin.
Dr. Thibodeaux posed two questions: What are the implications for people who received other organs from this donor, and should screening for HHV-8 be considered for all transplants?

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Vivianna Van Deerlin, MD, PhD, director of the molecular pathology laboratory at the Hospital of the University of Pennsylvania and professor of pathology and laboratory medicine at the Perelman School of Medicine, tells CAP TODAY that confirmation of donor origin of the tumor in this case will require additional testing, for example doing laser capture microdissection of the tumor cells and analyzing them because of the mixed nature of the cells and the difficulty in estimating the percentage of each histological component. “Further studies of this interesting case are underway,” she says.

“In my opinion,” Dr. Van Deerlin continues, “the main take-home point from this case is that identity testing can be a helpful adjunct test to help determine the origin of tumor after solid organ transplant. However, interpretation of the results is not always straightforward because the specifics of each case are unique and varied.”n

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