Tissue Is Still the Issue

The need for adequate tumor samples hampers even state-of-the-art diagnostics such as NGS—but some technologies require less tissue, improving oncology biomarker testing for pathologists, clinicians and patients.

David Moore discusses his experience and the results of a three-year NSCLC molecular testing audit.

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David Moore discusses his experience and the results of a three-year, now fully published NSCLC molecular testing audit.

As a histopathologist specializing in thoracic pathology, I work between a molecular diagnostics laboratory (which receives and tests samples of all cancer types from a large range of institutions, including both NHS and private laboratories), University College Hospital, where I report thoracic histopathology, and University College London, where I participate in non-small cell lung cancer (NSCLC) research.

Why is there so much discussion about NSCLC biomarker testing?

In NSCLC, the current standard biomarker tests are for EGFR driver mutations, ALK translocations and PD-L1 expression. There are additional biomarkers, such as BRAF, ROS1, and HER2, that are associated with approved or investigational treatments. So there are already multiple tests to be done—and with the rapid progress of precision oncology, it’s likely that we will soon need to test for yet more biomarkers in yet more tumors.

In your opinion, what are the advantages of next-generation sequencing (NGS) over other methods?

The majority of biomarkers in oncology are predictive; they forecast patients’ response to therapy. For a number of cancer types, it’s now standard of care to test for predictive genomic biomarkers—EGFR driver mutations in lung cancer, for instance. Using NGS technology, we can apply one test that covers a number of genes relevant to not just one, but a variety of cancers. Our 22-gene panel can be applied similarly to lung (EGFR), melanoma (BRAF V600E), and colorectal (KRAS) cases, so they can all go through the same workflow. Secondly, and even more importantly, NGS allows us to generate analytical and potentially clinically useful information for many more patients.

Can you elaborate more on the clinical benefit?

In our lab, we’re able to test cases with a limited amount of material or a relatively low tumor fraction; for example, endobronchial ultrasound biopsies (which are often 90 percent lymphocytes, 10 percent tumor) or 4–5 mm² lung biopsies. And that means we don’t have to subject those patients to repeat biopsies (and that, as a result, they receive conclusive results faster), unlike other NGS assays that require more tissue or tumor material. We can also apply NGS to samples with evidence of formalin fixation artifacts. A minority of cases exhibit a significant pattern of lower-level variants typical of formalin-fixation effects, which can interfere with analysis, meaning that variants detected in clinically relevant gene regions may not be genuine mutations. Using a single-gene testing method like PCR, we would never be able to see these potential false positives, but with NGS, we can see the “background effect” of overfixation and, based on that, recommend rebiopsy and retesting. The EGFR resistance mutation EGFR T790M is often a low-frequency “transition variant” like we see in formalin-fixation artifacts, so the ability to exclude that possibility in a T790M-positive sample is crucial to ensure the patient receives the optimal therapy.

What about the difference in panel size—is bigger necessarily better?

I can’t say that any one solution is “the best,” because they are different. Some are large, multigene panels, whereas others—like our 22-gene test—are more focused. The advantage of a large panel is that you gain information on a much wider range of genes. On the other hand, there’s the law of diminishing returns. The more data you generate, the less likely it is to be clinically relevant.

Importantly, not all NGS technologies are equally suited to each sample type. Our test, for example, requires a minimum of 5 percent tumor content and can be applied to very small biopsies; some others require more tissue and at least 20 percent tumor fraction, which is significant. For example, in our study cohort of nearly 3,000 samples from across the UK, we have a good, unbiased sample of lung cancer tissue specimens. Yet almost one-third of those cases would fall below the 20-percent threshold, making them impossible to analyze with some other panels. The result? Patients might miss opportunities to receive therapy that could benefit them.

“Using this limited 22-gene panel, we’re able to return some information on 83 percent of cases.”
So let’s hear about your data audit…

We have audited all of the NSCLC cases submitted to our laboratory over the three-year period from 2015 to 2017. Our starting sample pool included 2,976 cases, of which 7.8 percent were rejected (mainly due to <5 percent tumor cell fraction). Of those accepted, MGS analysis was successful in 94.9 percent (a 5.1 percent failure rate). Median turnaround time was seven days.

We have pulled quite a bit of interesting information from these data. We’ve divided the samples into categories by tumor cell fraction (5–20, 21–50, 51–75, and >75 percent tumor). We have also analyzed the reasons samples were rejected or failed analysis, and we have identified the number of cases with a recognized driver mutation in KRAS, EGFR, BRAF, NRAS, PIK3CA, or ERBB2 (HER2). In a number of cases with no evidence of a driver mutation, there was evidence of amplification in another relevant gene that might account for a genomic driver event.

We performed additional analyses on the 2017 cases by looking at those negative for not only EGFR, KRAS, BRAF, NRAS, PIK3CA, and ERBB2 driver mutations, but also amplification evidence—about 33 percent of all cases. We investigated how many of those cases had evidence of other somatic mutations that were likely to be cancer-specific. The most common in that cohort was TP53, found in half of that subset, which reduced the number of cases without any tumor-relevant mutation to only 17 percent. So, using this limited 22-gene panel, we’re able to return some information on 83 percent of cases.

Are all of those variants clinically significant?

Not all of them. But there are a number of findings that might make those patients eligible for ongoing clinical trials, even though there is no approved therapy available.

There is also the additional benefit of excluding false negatives. In those 83 percent of cases, we can be sure that they contained detectable amounts of tumor DNA, and therefore the chance of our having missed any actionable mutations due to insufficient tumor DNA is very low. If only one marker is analyzed—let’s say EGFR driver mutations, which have a prevalence of about 15 percent—then, in 85 percent of cases, we can’t be sure that we have analyzed adequate tumor DNA, and therefore we can’t exclude the possibility of a false negative result.

What is the usual cause of false negative or positive results?

Some samples just don’t contain enough tumor tissue, specifically the actual tumor cells. In theory, these should be excluded from analysis based on sample acceptance criteria; however, some centers only send us precut tissue “curls,” so we are unable to verify that they have assessed the tumor content accurately. If the tumor content is lower than our threshold, it’s a potential source of false negatives—and that’s why we recommend that centers send us tissue blocks. Additionally, DNA can be degraded and overfixed. Our technology is robust and sequencing is successful in 94.9 percent of cases, but there is always room for improvement in preanalytical handling procedures. We have identified some trends in our data that will be part of our upcoming publication, and we want that to contribute to awareness and education about this issue.

You mentioned acceptance criteria; what are those in your laboratory?

As a rule, we require 4 mm² of tumor area and minimum 5 percent tumor fraction, but there is some flexibility. We can sometimes test samples below 4 mm² or macrodissect a tumor out from cases that are <5 percent. Ideally, of course, we get the whole block—but we often receive slides (we have no minimum required number), or tissue curls that come in a tube. Such samples are clearly suboptimal because we can’t perform a proper preanalytical review.

And where can we see all the data?

You can find the data in the Journal of Clinical Pathology (http://dx.doi.org/10.1136/jclinpath-2018-205319)

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