# Colorectal Cancer Molecular Diagnostics Q&A

## Responses prepared by Mary P. Bronner, MD

Dear participants in the ARUP Colon Cancer Molecular Diagnosis webinar:

Since your post-webinar questions were all so good and I thought the answers might be useful for everyone, here are the answers to all the submitted questions. I hope these are helpful and thank you for your interest!

Q. Should gastric or duodenal adenomas be tested for MSI?

A. I think the answer here is yes. I don't recommend testing colonic adenomas unless they are unexpected (like in a young person) or from a high-risk family, simply because the pretest probability in regular old colonic adenomas is exceedingly low due to the extremely high prevalence of sporadic type adenomas (approaching 40–50 percent of patients older than 50 in the US). Conversely, sporadic duodenal and gastric adenomas are very rare outside of FAP and HNPCC, so I do think it is very worthwhile to screen them for MSI.

Q. What about OncoType DX?

A. I can't comment as this test is proprietary and tightly controlled by Genomic Health, a company that doesn't license this testing to other pathology labs. I have no experience with this test.

Q. Is there any indication of *KRAS* testing in CRC that are MSI-H by IHC? Are these abnormalities mutually exclusive?

A. *KRAS* testing for EGFR inhibitor therapy applies to all colon cancers, regardless of their MSI status.

Q. Do you recommend the shotgun approach to molecular testing,- MSI, *KRAS*, *BRAF* on all first-time CRCs or a stepwise approach (MSI, *KRAS* first, then *BRAF* if *KRAS* WT and/or MSI-H)?

A. I don't like shotgun approaches for anything, although many laboratories promote this approach for financial gain and ease of workflow. Our philosophy has always been to provide the most cost-effective and accurate testing algorithms for our patients and clients. We work with client laboratories to help them set up their own testing. This is also evident in our Analyzing Test Ordering Patterns™ (ATOP®) program at ARUP. Sometimes the more expensive test is the best choice to save money in the long run. Our ATOP program helps physicians monitor what they have ordered and the efficacy of their choices. An ATOP report identifies potential over-, under-, and misuse of individual laboratory tests and assesses the clinical and economic impact of suboptimal test ordering. This information can be shared by laboratory professionals with the clinicians who order tests for their patients. The insights gained from an ATOP report can be used to improve patient care, increase efficiency, and reduce costs.

Relative to colorectal cancer molecular testing, I think that all colon cancers should be tested for MSI—it is cost effective and has an enormous impact on patient care. The MSI algorithm I showed in my talk will then provide the most cost-effective and best diagnostic information for downstream testing in the MSI paradigm. I also think that all metastatic colorectal cancers should be tested for *KRAS*. If you

find a *KRAS* mutation (40 percent of all CRCs) then you're done and the patient should not be treated with anti-EGFR Rx. If you find the cancer is *KRAS* WT, then you really need to consult with the medical oncologist treating the patient. It is highly variable at this point in time whether oncologists want or will use additional genotyping data for the downstream potential modifier genes: *BRAF*, *PTEN*, or *PIK3CA*.

Q. Has your lab run into problems with the PMS2 IHC as far as variability in staining, even within one tissue block?

A. Yes, of the 4 ab's, I find PMS2 and MLH1 to be the most variable and tricky, but every lab is somewhat different. Interpretation of the IHC stains can be tricky (at least 10 percent of the time), and you really have to get experience with them to get good at them. Nuclear proteins like these are frequently more capricious in my experience. Harsh conditions such as on automated strainers may not work as well on these delicate nuclear epitopes. I have a very high threshold for interpreting these MMR stains as having a definitive loss of expression and want to be really certain before I interpret a case this way, especially given the major implications for the patient and their family. The control staining of PMS2 loss with MLH1 losses, and similarly MSH6 loss with MSH2, is a very useful control.

We have found that MMR IHC is tricky enough that it doesn't pay to try to save money by staining only a subset, as theoretically could be done. The frequent grayness of the staining and the help provided by having all four results to view in combination makes the upfront approach of staining all four the safest and best in my opinion. I have a very low threshold for calling the IHC MMR stains indeterminate/uncertain.

I think the MSI test is far more robust and black and white as a test, so I have no qualms in uncertain IHC cases regarding reflexing to MSI PCR testing for confirmation prior to diagnosing Lynch syndrome and then pursuing sequencing if there are at-risk family members to be screened by blood testing.

Why don't we then start with MSI PCR instead of IHC? The PCR test has the major drawback of not pinpointing which of the four genes is involved, so you have to do IHC testing anyway. Finally, even indeterminate IHC usually allows you to pick the most likely gene to go after by sequencing. Thus overall, we think IHC testing is the better place to start, but there are certainly good arguments for starting with PCR.

Q. Is there a sensitivity difference for MSI testing between PCR and immunoperoxidase?

A. Technically, yes, but practically, no. MSI may be slightly more sensitive, as not every *MMR* gene mutation leads to protein absence—thankfully, the great majority do, so that IHC loss of expression is virtually as sensitive as MSI PCR. Further, IHC gives you the advantage for pinpointing which gene to sequence in case you find a loss.

Q. Does it matter whether you use a small biopsy for MSI testing by immunoperoxidase, or should this be done on the larger resection specimen?

A. I think it is better, actually considerably better, to test pre-operative biopsies, because they work much better for the IHC stains (probably due to better fixation), and because you then give the clinicians info on Lynch syndrome prior to surgery. Most surgeons will do a total colectomy for Lynch rather than the subtotal approach for sporadic colorectal cancers.

Q. If a patient has *KRAS* wild type and is treated with cetuximab, do they only gain an additional two months expected survival difference?

A. Yes—isn't that terrible? The oncologists have decided this is still worthwhile, despite the high drug costs and the high toxicity. Reviewing more than eight trials for which I compiled data from patients treated with anti-EGFR Rx relative to *KRAS* genotype, the ranges of improved survival for *KRAS* WT over MUT are: progression free survival zero to five months and overall survival zero to seven months.

Q. Is there any particular type of thyroid cancer in Cowden's syndrome?

A. Mostly follicular cancer (as well as follicular adenomas).

Q. For *BRAF* V600E-positive colorectal cancers, is Zelboraf effective treatment?

A. While I'm not an oncologist and really can't comment, a quick literature search indicates promise for this agent.