

ADVANCES IN ONCOLOGY

Current Developments in the Management of Solid Tumor Malignancies

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Molecular Stool Sample Assays for Colorectal Cancer Screening

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H&O What is the rationale behind molecular stool sample assays as a way to screen for colorectal cancer?

BL Many people are hesitant to be screened for colorectal cancer because the standard approaches for doing so, such as sigmoidoscopy, colonoscopy, and radiologic tests, tend to be invasive and embarrassing. Therefore, any noninvasive test would be of value, simply because more people would be screened. Based on experience testing for hidden blood using stool samples, it is clear that some individuals are more comfortable with this approach.

The scientific rationale for the molecular stool sample assay is that tumor cells are shed into the lumen of the colon, where they become mixed with the bacteria, food contents, and water that constitute stool. It has been known for several years that it is possible to detect DNA mutations in stool samples using sensitive techniques. As new understandings of how colon cancer develops have emerged, more genetic targets that are related to this process have been identified. The aberrant DNA present in stool samples can be analyzed for the presence of these targets.

H&O Could you describe the initial development of the molecular stool sample assay for colorectal cancer screening?

BL This work began with Dr. Bert Vogelstein at Johns Hopkins University. In the 1990s, Dr. Vogelstein's then-junior colleague, Dr. David Sidransky, identified a technique that enabled the detection of mutated *ras* oncogenes in some stool samples of patients with colorectal cancer, some of which our group had provided. Once a mutation associated with a particular type of tumor has

been identified, we know what to look for in the stool. One of the advantages of the *ras* oncogene in terms of scientific research is that the mutations are confined to only 2 codons, which makes screening for the mutation very feasible.

The early studies were not blinded. The investigators developed a method to purify the DNA from stool samples knowing what they were looking for, an approach that is quite different from typical screening studies. In a blinded screening study, there is no foreknowledge of what the abnormal genes might be, and the investigators use a panel of markers to see which might be present in the stool. Such studies depend on the prevalence of certain abnormalities that are common in many tumors.

H&O Why was the *ras* oncogene selected for these studies?

The decision to focus on the *ras* oncogene was part of the evolution of the knowledge of how colon tumors progress from a normal epithelium to an adenoma and then into cancer. The *ras* oncogene is a very common mutation present in this process.

H&O According to the early and subsequent findings, what are the advantages and disadvantages of the molecular stool sample assay approach?

BL One of the advantages of this technique is that it is relatively effortless. The patient needs only to provide a sample that, in the current form of the test, is deposited in a special box containing a medium that preserves the integrity of the DNA. From a practical point of view, this assay is easy to perform.

The present cost of the commercially available test is relatively high—in the range of \$300–\$500—and this is certainly one of the disadvantages. Also, in recent large-scale studies of this approach, the overall sensitivity was just over 50%; however, the specificity was very high, approximately 96%. In other words, there were relatively few false positives associated with this screening technique,

but quite a few cancers and adenomas were missed.

H&O Based on these findings, what is the most practical application of this approach right now?

BL The value of the molecular stool sample assay currently is related to people who would not otherwise be screened. Someone who is unwilling to undergo a colonoscopy may be willing to provide a stool sample and pursue further testing if the assay is positive. The molecular approach does not yet meet the criteria for large-scale screening because a significant lesion would be missed at a rate of approximately 50%.

H&O Is the technique being improved?

BL Yes. Efforts are underway in several directions. The efficiency of DNA extraction is being improved, and a more sensitive technology for the existing markers has been developed by the company (Exact Sciences) that makes the currently available test. The company is also working with other investigators who have identified additional markers that could be incorporated into this stool-based assay, as well as with Dr. Vogelstein who is developing other novel detection techniques. Also, it has been found that one of the reasons why the results of the large-scale trials were not strongly positive is that the long DNA that was previously used as a marker was degraded during transportation. Ways to overcome this have been found. All of these efforts and investigations are contributing to enhancing the sensitivity of the assay while maintaining its high specificity. It is likely that a significantly better methodology will be available soon, perhaps within the next 6–9 months.

H&O How might an improved molecular stool sample assay be incorporated into the current approaches for colorectal cancer screening?

BL To answer this question, it would need to be understood how this test compares with other noninvasive screening techniques. There are proprietary products currently available that seem reasonably competitive, such as fecal immunochemical testing, which looks for hidden blood in the stool and is employed in the United States, Japan, and Australia.

The original studies that evaluated the efficacy of testing for hidden blood in the stool were based on program analysis, which involved yearly repetition of the test. With the DNA-based test, the only results that are available are from a one-time analysis; we do not yet know how effective the technique might be if it were repeated every 2 or 3 years. In addition, the cost of performing the test on a repeat basis is not known. Cost is a central issue relating to how this technique might be incorporated into

standard screening practices. As was mentioned earlier, this test might be best used as a means of deciding who needs to undergo colonoscopy. However, if the cost of the DNA-based test were the same as that of a colonoscopy, then one may want to proceed directly to colonoscopy. If the DNA-based test were available for \$100–\$200, then it would be reasonable to perform this test prior to a more invasive procedure. Ideally, the test would need to be more sensitive, perhaps at the level of 80%, in order to warrant the cost.

Thus, there are 3 main factors that will determine how the DNA-based test will be used: patient preference, cost, and what other tests are available. Clinicians need to monitor the progress of the available molecular stool sample assays as well as other technologies being developed, such as virtual colonoscopy. That technique is also undergoing development, and a less extensive bowel preparation, as has been required with traditional colonoscopy, will likely make this a popular method of screening.

H&O Are there other tests on the horizon that appear promising?

BL One intriguing possibility is that instead of using a stool-based test, it may be possible to detect genetic abnormalities in the blood. Obviously, many people would prefer a blood test to any other option. However, this approach could not be used as a complete screening method. To reduce the impact of colorectal cancer, finding early, curable cancers and adenomatous polyps is crucial, because removing the latter can prevent colon cancer from ever developing. Stool-based tests can detect both polyps and adenomas to varying degrees because both shed abnormal genes into the stool. A blood test would likely detect a cancer-related gene abnormality, but would most likely not be effective for detecting an adenoma, because the gene products from a benign lesion are not thought to be shed into the blood. A blood-based screening test would be effective for someone with cancer, but a stool test would still be necessary for detecting adenomatous polyps.

Suggested Reading

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