For tumor markers, hope springs eternal

William Check, PhD

Devotees of Eastern mindfulness practices such as meditation and yoga believe it is possible to attain self-awareness and enlightenment, or satori, in this life. Not that it’s easy, but that it’s a goal that can be reached through intense effort and focus. Those who search for specific and sensitive serum tumor markers also believe they can reach their goal with focused thought and hard work. But just as most people who seek enlightenment fall short, so, too, most tumor markers end up being helpful, but not ideal, as screening tests.

Nonetheless, as new molecular methods have arisen, particularly microarray gene expression analysis, those who seek serum tumor markers continue their quest with renewed enthusiasm. With tumor markers, as with satori, reality is a mixture of compromise and hope.

“Most classical biomarkers that we use today in the clinic—which were developed 20 years ago or more—are actually not very good for diagnosis but are useful for monitoring therapy and predicting response,” says Eleftherios P. Diamandis, MD, PhD, FRCP, head of clinical biochemistry at Mount Sinai Hospital, University Health Network, and Toronto Medical Laboratories and professor and head of the Division of Clinical Biochemistry, Department of Laboratory Medicine and Pathobiology, University of Toronto. “There has always been a lot of interest in finding markers that are good for diagnosis and especially for screening, identifying latent disease in asymptomatic individuals,” continues Dr. Diamandis, who organized and will speak at a course this month at the AACC annual meeting on the use of tumor markers in the clinic and new developments. “Unfortunately, we have never had a very good way to screen for cancers, with the possible exceptions of the Pap smear, mammography, and prostate-specific antigen.”

In the past five to 10 years, with the completion of the Human Genome Project, many people became excited again about finding effective diagnostic and prognostic markers. “Some people said now that we know the genes that encode proteins we should be able to study them systematically,” Dr. Diamandis says. “That is a reasonable way of thinking.” He identifies a second recent driving force for biomarker discovery: new analytical technology, chiefly gene expression microarrays and mass spectrometry (Li J, et al. Clin Chem. 2005;51:2229–2235). Researchers expected that combining knowledge about gene sequences with new technologies would make it possible to discover new biomarkers. But Dr. Diamandis adds an important caveat to use of the new technologies: “With these methods you are looking at many molecules at a time,” he says. So you have to add bioinformatic approaches, such as neural networks and logistic regression analysis, to interpret the large data sets from these multiparametric technologies.

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“That is why a lot of granting agencies, biotech companies, and pharma companies jumped into this biomarker extravaganza,” Dr. Diamandis says. “You can find a biomarker now for just about any tumor.

“So what has happened in reality?” he asks. “Are biomarkers coming out of our ears?” The truth, in his view, is that though many papers have proposed novel methods for diagnosis and prognosis, few of these methods have proved out. “Unfortunately, the pipeline for infusion of biomarkers into the clinic today is dry,” Dr. Diamandis says. “No major biomarker has been introduced for cancer over the last 10 years, with the possible exception of HER2 testing to predict response to Herceptin in breast cancer.” And, he points out, the discovery of overexpression of HER2 in cancers dates back 30 years. “It came to clinical attention only in the last five years because of the development of a therapeutic monoclonal antibody,” he says.

The validation stage has been the “big disappointment,” Dr. Diamandis explains. “People who tried to validate the proposed markers found that all these methods had intrinsic biases and appear not to be as good as initially claimed.” For example, a meta-analysis published in 2005 that tried to validate seven prognostic algorithms derived from microarray expression profiling found that “the list of genes identified as predictors of prognosis was highly unstable; molecular signatures strongly depended on the selection of patients in the training sets” (Michiels S, et al. Lancet. 2005;365:488–492). In general, Dr. Diamandis says, “overtraining” of the computer introduces artifact and bias. Investigators select half of the patients for the training set, then apply the resulting algorithm to the other half, the test set. “Overtraining means you carefully select what you tell the computer,” he says. “So the algorithm is very competent at finding minute differences between tumors and normals in your patient group.” However, the test set is not truly independent of the training set. “Once you take the algorithm into a new group of patients,” he says, “then it is not as versatile.” (For other critiques of microarray methods, see: Diamandis EP. Cancer Res. 2006;66: 5540–5541; Check E. Nature. 2004; 429:496–497).

He describes the current state as one of confusion. “There are lots of new technologies and lots of proof of principle papers, but no continuation to the validation stage,” Dr. Diamandis says. What we need, in his view, is rigorous followup to work that has already been published to see if the conclusions of the first papers were actually correct.

Despite these obstacles, Dr. Diamandis is hopeful for the future. “The tools we have for discovery today are extremely powerful. Genetic markers may give us a chance to identify cancer predisposition even before the disease appears. So the prospects are very good,” he says. One promising approach is to detect minute changes within the genome, such as single-nucleotide polymorphisms. “SNPs may provide a very good tool for detecting predispositions to various cancers,” he says.

One company that is hopeful about genetic approaches is ChondroGene Inc., of Toronto. Daniel H. Farkas, PhD, HCLD, vice president of clinical diagnostics, notes that over the past 15 years commercial gene chips have expanded in capacity and are now able to analyze tens of thousands of genes. “There are now many examples of the fact that gene expression differs between normal and cancer tissue,” Dr. Farkas says. He cites breast cancer and lymphoma as two outstanding cases.

It is Dr. Farkas’ impression that gene profiling has become more accepted as a clinical tool with the recent introduction of tests for cardiac transplant rejection (AlloMap) and to predict the probability of recurrence in node-negative breast cancer (Oncostype Dx). ChondroGene was founded on initial observations that gene expression profiling of blood differentiates individuals who do or do not have osteoarthritis. That principle, the Sentinel Principle, was extended to cancers, and the company intends to go to market first with a test for colon cancer. Dr. Farkas says this assay will be done on blood, rather than biopsy tissue, which, he predicts, will get more uptake from the pathology community as well as patients. Consonant with its broader orientation, the company’s name will soon change to GeneNews, Ltd.

While the search for ideal tumor markers with advanced methods proceeds, a few conventional, less-than-ideal but practically helpful markers were introduced recently. One is an assay for screening those at risk of mesothelioma. Another seeks to refine an existing marker for hepatocellular carcinoma.

About 1500 km from the office of
Bruce W.S. Robinson, MD, FRACP, FRCP, at the University of Western Australia in Perth, is a large deposit of blue asbestos, the most deadly form of asbestos. Because of mining there, the state of Western Australia has the highest incidence of malignant mesothelioma in the world. Dr. Robinson and his colleagues follow a cohort of 2,500 people who were exposed to asbestos.

“People come and see us once a year,” says Dr. Robinson, who is professor of medicine in the School of Medicine and Pharmacology and consultant respiratory physician, Department of Respiratory Medicine, Sir Charles Gairdner Hospital.
One goal of the follow-up program is to explore markers for early detection. Five years ago Dr. Robinson read work by Ingegard and Karl-Eric Hellstrom at the University of Washington reporting high levels of a protein called soluble mesothelin-related peptides, or SMRP, in ovarian cancer. “I contacted Ingegard and said, ‘I have all these patients with mesothelioma, which is probably a better disease to study.’ She said send me a half-dozen samples.” Dr. Robinson did. All the samples had very high levels of SMRP.

In a subsequent study of 40 healthy, asbestos-exposed individuals, seven had elevated levels of SMRP. Over the eight years following sampling, three developed mesothelioma, compared to none with normal levels of SMRP (Robinson BW, et al. Lancet. 2003;362:1612–1616). Dr. Robinson calls those results “encouraging” but cautions, “That doesn’t mean it’s a good screening test. We still have to determine the false-positive rate. It’s quite hard to produce a good screening test.”

Right now Dr. Robinson considers the SMRP assay useful in two ways. First, it can help with diagnosis in someone who presents with suspicion of the disease. “If an asbestos-exposed patient comes in with pleural effusion, measuring mesothelins is a very good idea,” Dr. Robinson says. “If it is positive, there is not much else it could be. We use it all the time.” They measure it in pleural fluid as well as serum.

Second, it can be used for monitoring response to treatment and predicting poor outcome. “It’s a better marker of relapse than imaging,” Dr. Robinson says. He adds, “We hope it might be useful as a screening tool for people at risk of mesothelioma to allow early diagnosis and treatment. But better detection is useful only if it makes a difference, so we must show that it leads to better treatment outcomes.”

Ongoing work on SMRP’s place in screening is taking place in the laboratory of Petra Stieber, MD, head of the diagnostic oncology research laboratory in the Institute of Clinical Chemistry at the Grosshaderm Clinic of the Ludwig-Maximilians University, Munich. Evaluating potential new markers is one of the jobs of Dr. Stieber’s laboratory, which has access to a variety of serum samples from patients with all types of cancers and nonmalignant diseases.

“We look at all diseases in a global way,” Dr. Stieber says, “and we work in blood, not in tissue. If you work in tissue, you know where the sample comes from. In blood we lose any context to organ specificity.” In this regard, Dr. Stieber follows the guidelines of the European Group on Tumor Markers, which she calls “the first group to say that if you are working in blood you have to respect all kinds of diseases.”

As with all tumor markers, SMRP is not tumor specific. “It is present in all blood from birth, which is true of all actually used biomarkers,” Dr. Stieber says. “Up to now we don’t have a single tumor-specific biomarker. So we can’t establish a diagnosis using any single marker. We compared SMRP to all other oncologic biomarkers that are today relevant in lung cancer.” These included carcinoembryonic antigen (CEA), squamous cell carcinoma antigen (SCCA), progastrin-releasing peptide (ProGRP), cytokeratin fragment 19 (CYFRA 21.1), and neuromune specific enolase (NSE), all of which are recommended for the differential diagnosis of lung tumors in the guideline of the National Academy of Clinical Biochemistry, for which Dr. Stieber is the chairperson.

As expected, there was overlap in SMRP values between healthy individuals and those with mesothelioma, as well as patients with many other diseases. However, the highest values were all found in mesothelioma patients. “No other tumor disease leads to this significant release,” Dr. Stieber says. At this time she is unwilling to recommend a cutoff value for positivity. Nor is it possible to establish an ROC curve. “We are not at the end of our investigation yet,” she says. “We are still in the middle.”

However, Dr. Stieber does think that SMRP will probably be useful in a panel of markers to establish tumor type in a patient with a known lung tumor, which can reduce the need for biopsy. “Lung tumors have many different histologies,” she says. “There is no other tumor with this complex range.” The biomarker pattern can help with the diagnosis. In small cell lung cancer, for instance, ProGRP and NSE are elevated. Similarly with CYFRA 21.1 and SCCA in squamous cell cancer. “My hope is that a significant increase of mesothelin would point us toward mesothelioma,” Dr. Stieber says.

“The main question that moves the world today is screening,” she continues. “We would like to have a screening test in blood to detect mesothelioma patients in an at-risk group.” That would be done with kinetics of SMRP increase, since people at risk are followed regularly. Verifying this application of SMRP would require following patients over years in mesothelioma and in other diseases. “This is not so easy to do,” Dr. Stieber says.

Rights to SMRP as a diagnostic test were acquired from the Hellstroms by Fujirebio Diagnostics Inc. of Malvern, Pa. (formerly Centocor Diagnostics) under the name Mesomark. Mesomark is available outside the U.S. and is now before the FDA, says Jeff Allard, PhD, Fujirebio’s chief scientific officer. Since there are only 2,000 to 3,000 new cases of mesothelioma each year in the U.S., and per-
haps 10,000 to 20,000 worldwide, Fujirebio is working with the FDA to develop Mesomark as a humanitarian device, a designation equivalent to orphan drug or compassionate use status. In this category, the test would be approved based only on safety data, not on efficacy, and would be available under protocol, probably only in a limited number of centers. According to Dr. Allard, Mesomark would be ordered for patients with diagnosed mesothelioma to detect recurrence earlier and to determine response to therapy—which, Dr. Allard says, “in this case is not a lot.” Primary treatment is extrapleural pneumonectomy—resection of the lungs, peritoneum, and diaphragm. Many patients are inoperable. “Screening is not an appropriate use right now,” Dr. Allard says, “but we are hopeful that will be the case.” Data on Mesomark’s discriminatory power have been published (Scherpereel A, et al. Am J Respir Crit Care Med. 2006;173: 1155–1160).

A second, newer test for a circumstance application, this one FDA-approved, is Wako Diagnostics’ AFP-L3% kit, to help determine the risk of developing hepatocellular carcinoma, or HCC, in subjects with chronic liver disease (cirrhosis). More than 90 percent of primary liver cancer occurs in patients who have cirrhosis. In Asia the primary cause of cirrhosis is hepatitis B virus infection; in the U.S. it is hepatitis C virus infection with or without alcoholism. Richard Sterling, MD, professor of medicine in the section of hepatology and liver transplantation at Virginia Commonwealth University Health Systems, Richmond, notes that in a person with cirrhosis the chance of developing new liver cancer is two to eight percent annually. “HCC has one of the highest death rates by site,” he adds.

Dr. Sterling and colleagues have generated retrospective evidence that picking up liver cancer in a surveil-

lance program improves outcomes. In their registry of 269 liver cancer patients, those with known cirrhosis who were in a surveillance program had better three-year survival than those not known to have cirrhosis. Better survival probably reflected a higher rate of liver transplantation—the preferred treatment for HCC—in those enrolled in the program.

Surveillance has traditionally focused on abdominal imaging every six to 12 months by ultrasound, which is relatively inexpensive. Measuring alphafetoprotein, or AFP, in blood has also been recommended. However, Dr. Sterling says, many people with liver cancer have normal AFP values, and some with chronic hepatitis and advanced scarring of the liver without cancer have elevated values. “So AFP alone is neither sensitive nor specific enough for clinical practice,” he says. Workers in Japan found that a subfraction of AFP with a distinct glycosylation pattern—AFP-L3—could help in this situation. “At least in Japan, an elevated proportion of AFP in the L3 subfraction [AFP-L3%] was associated with more vascular invasion, shorter doubling time, and worse survival,” Dr. Sterling relates. When the Japanese workers followed people over time, AFP-L3% appeared to augment total AFP in determining who would develop cancer.

Dr. Sterling led a subsequent seven-site North American study in which 332 patients with chronic hepatitis C-related cirrhosis were followed with AFP, AFP-L3%, and liver imaging every three months up to 24 months, with the endpoint being diagnosis of HCC. “AFP-L3% had clinical utility in this setting,” Dr. Sterling says. Those with elevated AFP-L3% were more likely to develop cancer. And a normal AFP-L3% value increased specificity in persons with mild-to-moderate total AFP elevation. Dr. Sterling declined to give details because the data have been submitted for presentation at a fall meeting. In an analysis of another data set, Dr. Sterling found that AFP-L3% increased specificity from 85 percent to 92 percent in patients with total AFP >20 ng/mL.

How this test will affect management is still being worked out, he says. Between one-third and one-half of people who develop liver cancer have normal total AFP; their cancers are detected only on imaging. Since AFP-L3% is unmeasurable in those with normal total AFP, the test will have limited utility in this group. Dr. Sterling’s opinion is that AFP-L3% will be most useful in a surveillance program in patients who have no mass on imaging and elevated total AFP. Low AFP-L3% in this setting can be “somewhat reassuring,” he says, and may reduce the frequency of expensive imaging tests. However, he adds, “I don’t have the numbers to do an analysis to see whether doing this test can save you money by avoiding an MRI.” That depends on the cost of the test. There is also an indication that those with a normal imaging test and elevated total AFP are at two- to threefold higher risk of developing cancer if AFP-L3% is also elevated.

Further data will come from the HALT-C study, in which people with hepatitis C being treated with maintenance interferon are followed for liver cancer, transplantation, or death. AFP-L3% and another investigative marker, des-Á-carboxy prothrombin, are being measured prospectively.

Measuring AFP-L3% may also be useful in predicting a worse prognosis in patients with HCC, including recurrent disease after liver transplantation and survival after medical or surgical interventions, says Robert Gish, MD, medical director of the liver transplant program and chief of the Division of Hepatology and Complex GI at the Physician Foundation at California Pacific.
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ic Medical Center, San Francisco. It may also be useful as an adjunct in diagnosis.

Dr. Gish cites abstracts presented at this year’s Digestive Diseases Week meeting to characterize the value of AFP-L3%. In a retrospective study from the University of Pittsburgh, sensitivity of AFP-L3%, des-Á-carboxy prothrombin, and AFP for unresectable HCC was 61.6 percent, 72.7 percent, and 67.7 percent, respectively—approximately equivalent. (Combining markers increased sensitivity to 86 percent; specificity was not given.) In multivariate analysis, AFP-L3% was the only test that showed a difference in outcome, Dr. Gish notes. High AFP-L3% levels also predicted portal vein invasion. “So this test is helpful to determine prognosis,” he says. Work from the Mayo Clinic showed that AFP-L3% may have value in patients with indeterminate total AFP values—between 10 and 200 ng/mL. In this group, AFP-L3% greater than 35 percent had 100 percent specificity, though only 33 percent sensitivity, supporting the contention that a normal AFP-L3 could provide assurance that a negative test would indicate a very low probability of the presence of HCC. (This work did not find an association between L3% and vascular invasion or survival.)

In clinical practice, Dr. Gish says that, because a high AFP-L3% may indicate a worse prognosis (portal vein invasion or recurrent disease), he and his colleagues may decline a patient for liver transplant if the patient’s HCC size and number are outside transplant criteria and L3% is high. Also, stepped-up surveillance after resection or ablative therapy in patients with high AFP-L3% may be appropriate. For diagnosis, in a patient with a negative scan and high total AFP, a high AFP-L3% indicates a higher likelihood of HCC. “We might go to MRI in this case,” he says. “Where-as, if the L3% is negative, we would probably less frequently use imaging and monitor over one to three years.”

Though useful, AFP-L3% may be used with or displaced by des-Á-carboxy prothrombin. A Digestive Diseases Week poster from the University of Michigan concluded: “DCP was more sensitive and specific than AFP and AFP-L3 for the diagnosis of early stage HCC, especially in patients with hepatitis C.”

Turning to gene-based tests, Dr. Diamandis points to Gen-Probe’s urine-based Aptima assay for mRNA from the PCA3 gene. At this point the analyte-specific reagents for PCA3 have been validated by AmeriPath and Bostwick Laboratories and are commercially available. In data presented at the 2006 meeting of the American Urological Association, one group measured the ratio of PCA3 to PSA mRNA in a diverse sample of patients. Sensitivity was 63 percent and specificity 82 percent at an optimal cutoff ratio. For comparison, the specificity of the serum PSA assay was 42 percent. This new assay may prove useful as an adjunct test. Gen-Probe is seeking FDA approval.

Dr. Farkas says ChondroGene’s simple, real-time PCR-based tests are based on the Sentinel Principle, which postulates that blood cells can act as sentinels of disease and thus provide a source of gene transcripts for detecting pathological conditions (Liew CC, et al. J Lab Clin Med. 2006;147: 126–132). To develop a test for colon cancer, the company’s scientists started with hundreds of genes that characterized the expression profile of colon cancer and now have five that maintain the cancer/no cancer distinction.

In work presented at this year’s meeting of the American Society of Clinical Oncology, company scientists reported using gene expression profiling of 31 blood RNA samples to identify differentially expressed genes. Quantitative real-time PCR of 115 samples then identified a subset of five genes that achieved sensitivity of 98 percent and specificity of 51 percent in the training set. This five-gene combination was then used in a blinded test of 83 samples, achieving sensitivity of 95 percent and specificity of 42 percent. “We are still working on which genes to include in the panel,” Dr. Farkas says. “We would like not only to differentiate cancer from no cancer, but also adenomatous polyps and pre-cancerous lesions from the other two states.” ChondroGene scientists are working with several institutions to get more cases and contemplating large-scale clinical trials.

Speaking as a former laboratory director, Dr. Farkas says a test for colon cancer that focuses colono-scopies more efficiently would be clinically helpful. The test would be offered to those over age 50 who are going to get a colonoscopy. “You would want 90 to 95 percent sensitivity,” Dr. Farkas says. (For use in those under 50 years, sensitivity would need to be even higher.) “You could live with a bit lower specificity.” Those who test positive would proceed to colonoscopy; those with a negative test result would not. Dr. Farkas can imagine a physician telling a patient, “You don’t have a family history, you don’t have symptoms, and you have a negative PCR result. You don’t need a colonoscopy now. Come back in one or two years.” He can also see third-party payers being interested in a $300–$400 test that would spare a $1,200–$1,300 procedure, though the net financial impact would depend on how many tests would need to be done to prevent one colonoscopy.

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More-speculative gene-based methods are under investigation. Dr. Diamandis terms one “maybe a breakthrough in the cancer biomarker field,” but cautions that it is “extremely early.” Typically chromosomal translocations are thought to be almost exclusively confined to leukemias and lymphomas. Recently a group of investigators showed that they are common in prostate cancer (Tomlins SA, et al. Science. 2005;310:644–648). “Now that’s revolutionary,” Dr. Diamandis says. “If you can detect the translocations, they may form the basis for a diagnostic test.”

Even more speculative is measurement of free DNA. “People have demonstrated clearly that there is free DNA in the serum of cancer patients,” Dr. Diamandis says. “Many people now believe that methylation of DNA may be a very good marker of malignant disease.”

Given all these possibilities, Dr. Diamandis says it is mostly a matter of time and effort before better diagnostic tests arise.

“People have rushed to make claims that are not reproducible,” he says. “But the future should be pretty bright.” It will take more time to validate what is published to make sure the data are robust and reproducible.

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