

# GENE-BASED DIAGNOSTICS: READY FOR PRIME TIME?

The rush is on to develop gene-based diagnostic tests that will detect diseases earlier and predict response to drugs and treatment. But the road to widespread use by physicians may be a long one.

BY KATHERINE T. ADAMS, *Senior Editor*

Malcolm Gladwell seems to have gotten it right when he describes the tipping point (in his book of the same name) as that moment in time when incremental increases in understanding combine and ignite a knowledge explosion that creates change. That point has arrived in the world of gene-based diagnostic testing.

The number of tests in development — and the number on the verge of being marketed — is growing exponentially. When added to tests already in use, the burden on physicians to assimilate them into clinical practice could be overwhelming. The good news, of course, is that these tests, in time, will change the standard of care for patients who suffer from debilitating and life-threatening diseases.

Sparked by the 2003 publication of the mapped human genome, the U. S. market for genetic diagnostic tests and reagents, excluding infectious disease tests, will reach \$1.5 billion by 2011, estimates industry consultancy Frost & Sullivan.

Interest in gene-based testing, particularly in tests that have already reached market, is high, says

Diane C. Robertson, director of health technology assessment services at ECRI, the Plymouth Meeting, Pa.-based health services research firm. Among the tests currently generating the most requests for information is Genomic Health's Oncotype DX assay for diagnosing breast cancer and DNA tests for colorectal cancer.

Introduced in 2004, Oncotype DX is a noninvasive test performed on tumor tissue removed during initial surgery on women with breast cancer, and quantifies the likelihood that the disease will recur within 10 years and the potential benefit of chemotherapy. Results of a large validation study were published Dec. 30, 2004, in the *New England Journal of Medicine*. Genomic Health, in the midst of an initial public offering, was not available to comment for this story.

Robertson notes the lack of published evidence addressing the clinical utility of this test, developed in Pittsburgh by oncologist Norman Wolmark, MD, and colleagues. Although information has been published in abstracts and presented at meetings, Robertson says, no controlled trial has been completed to tell practicing physicians how this

test will change patient management.

"That's really the question," she says. "It's very appealing to doctors who want a tool to manage patients and to know whether they should give them chemotherapy. It speaks to the fact that chemo is a very crude form of treatment. Clinicians are grasping at any tool available to help put patients on a track that is most beneficial."

Michael S. Watson, PhD, executive director of American College of Medical Genetics, shares Robertson's view of the downside of how most drugs are administered today without any genetic-based knowledge about who might most benefit. "With most current drugs, we throw a bowling bowl at a disease — because the drugs haven't been individualized — and we throw it at everybody regardless of how a person might respond," Watson says.

**"In transplantation,** the most important thing cardiologists want," says Pierre Casigneul, XDx chief executive officer, "is an earlier signal than biopsy that there may be damage to the heart so they can intervene. It would also make sense economically."



## DIAGNOSTIC TESTS

“Some percentage will respond well, but we never know why the others don’t.”

Here are three tests (and a few in development) that are helping shift the metaphor from bowling balls to darts.

### QUANTIFYING DRUG METABOLISM

Roche Diagnostics’ CYP450, now cleared by the U.S. Food and Drug Administration for diagnostic use, is a DNA array or ‘gene chip’ that targets the cytochrome P450 complex. Based on Roche’s patented polymerase chain reaction (PCR) technology, the P450 looks for variations in two genes, CYP2D6 and CYP2C19, which influence drug metabolism. Lab analysis of a patient’s blood sample indicates — within eight hours, says Roche — if a patient is a poor, rapid, slow, or somewhere-in-between metabolizer. Roche estimates that 20 to 25

percent of commonly prescribed beta blockers, antidepressants, antipsychotics, proton pump inhibitors, and cancer-treating drugs, such as tamoxifen, are on the 2D6 and 2C19 genes or substrates.

Although much is known about tests like CYP450 through their use in drug development, “What’s missing is a correlation between studies and clinical use,” says Walter Koch, PhD, Roche Molecular Diagnostics’ Head of Research. “For example, if you have variation A, and you’re prescribing drug B, you need to adjust the dose for, let’s say a male patient weighing 200 pounds, by so much. That’s what’s still coming down the pathway.”

The test was approved for use in Europe in January 2005, but, Koch says, it’s too early to say how widely it is being used. “As with any new technology or test, there’s always a lag period before you get broad uptakes. More healthcare provider ed-

ucation will be required before that can happen.”

Teaching physicians how to use genomic tests is an overriding need, Koch admits, because it’s clear to him that clinicians have not been trained to fully appreciate the usefulness of pharmacogenetic information. He recognizes that although tests such as the CYP450 provide a particular result that predicts a person’s intrinsic enzymatic status or ability to metabolize drugs, they don’t provide specific guidelines on how to adjust drug dosages.

“Some publications in the scientific literature, particularly in the area of antidepressants, have begun to make those recommendations,” he says. “For example, if you are a poor metabolizer and you’re taking a tricyclic antidepressant, you might start with only 20 percent of the normal dose. But, by and large, that kind of information is not yet widely available.”

Roche is working with other pharmaceutical companies to establish standardized protocols so that the testing process will eventually become routine for physicians. In the short term, Koch says, CYP450 works best with patients who are not responding to a medication. “In the long-term, the test has the potential of moving in advance of prescription. Theoretically, it could be performed once because genes don’t change. But dietary factors — like eating a lot of grapefruits — and ingesting chemicals of various kinds can affect how you metabolize a drug. Physicians have to take all this information into consideration when making their dosing decisions.”

Another promising chip-based

### SNAPSHOT OF A KNOWLEDGE BOOM

The GeneTests Web site is a free medical genetics information resource for physicians and other healthcare providers and researchers, administered by the University of Washington, and funded by the National Institutes of Health. The site, updated weekly, features a home page that keeps a running tally of the scope of research into gene-based diagnostic testing.

- 317** GeneReviews (expert-authored, peer-reviewed, current disease descriptions that apply genetic testing to the diagnosis, management, and genetic counseling of patients and families with specific inherited conditions)
- 1,063** Clinics
- 585** Laboratories testing for
  - 1,171** Diseases
  - 878** Clinical
  - 293** Research only

SOURCE: GENETESTS: MEDICAL GENETICS INFORMATION RESOURCE, UNIVERSITY OF WASHINGTON, SEATTLE. INFORMATION CURRENT AS OF NOV. 7, 2005.

«[www.genetests.org](http://www.genetests.org)»

assay in the works is Roche's Ampli-Chip P53 for resequencing the P53 tumor suppressor gene, the most commonly mutated gene in all cancers. When this gene is mutated, Koch explains, it is generally associated with a poor prognosis because the gene product plays a critical role in cell cycle regulation as well as response to chemotherapy.

"When one looks at cancers that have mutations versus those that don't, you can get good prognostic information," he says. Patients with mutations are less likely to have a favorable outcome; however, if that information can be used to treat a patient more aggressively with a particular kind of chemotherapy or medication, that will likely change

the prognosis. The cancers of interest are lung and bladder cancer, but it's also been shown to be relevant in breast cancer."

Roche is classifying the molecular structure of all the various leukemias, currently done with seven or eight different tools or techniques that take up to two weeks to produce a result. Some forms of

## DIAGNOSTIC TESTS AVAILABLE AND ON THE HORIZON

Disease	Product	Manufacturer	Indication	Availability
<b>Bladder cancer</b>	UroVysion	Abbott Laboratories	Detects genetic changes in bladder cells in urine specimens using fluorescence <i>in situ</i> hybridization (FISH).	01/05
<b>Breast cancer</b>	InSite Her-2/neu kit	BioGenex	Breast cancer type 1 susceptibility protein. This <i>in vitro</i> diagnostic kit is used to identify breast cancer patients eligible for treatment with the cancer drug trastuzumab (Herceptin).	01/05
<b>Congestive heart failure</b>	NT-proBNP assay	Nanogen	Measures levels of N-terminal brain natriuretic peptide (NT-proBNP). Clinical studies indicate that the risk of mortality from congestive heart failure can be more accurately ascertained if levels of NT-proBNP are known.	06/05
<b>Cystic fibrosis</b>	Tag-It cystic fibrosis kit	Tm Bioscience Corporation	Cystic fibrosis transmembrane conductance regulator. FDA approved Tag-It based on a manufacturer study of hundreds of DNA samples showing that the test identifies the CFTR gene variations with a high degree of certainty.	05/05
<b>Hepatitis C</b>	Hepatitis C Virus Encoded Antigen (HCV Encoded Antigen/Enzyme Immuno Assay)	Abbott Laboratories	Detects hepatitis C virus encoded antigen	07/04
<b>HIV</b>	Multispot HIV-1/HIV-2 rapid test	Bio-Rad Laboratories	For detection and differentiation of circulating antibodies associated with HIV-1 and HIV-2 in human plasma and serum, as an aid in the diagnosis of infection with HIV-1 and/or HIV-2	11/04
<b>Sepsis</b>	ST In-Check Lab-on-Chip	STMicroelectronics and Mobidiag	DNA-based detection of sepsis-causing bacteria	Late 2006
<b>Tuberculosis</b>	DiagnostIQ	Proteome Systems	Antigen-based diagnostic test	Late 2005

SOURCE: SEC FILINGS, COMPANY WEB SITES

— Compiled by Tony Berberabe, MPH

leukemia have specific chromosomal rearrangements that lead to a specific treatment. Novartis' imatinib (Gleevec) is given only to those patients who carry a T922 translocation — the so-called Philadelphia chromosome, named for the city where it was discovered in 1960 — and it creates a new tyrosine chimeric product that is the target for this drug, Koch says. "This is an area where the molecular etiology of the disease helps to dictate specific, tailored treatments for that cancer."

Roche also is exploring early detection assays for cancer. "We are looking at DNA methylation markers — which are uniquely found in certain cancers — and asking, 'Can we find these markers in the blood of individuals long before their cancer shows up?' The first application where we are having reasonable success, and it's still early, is in colorectal cancer."

### WILL A NEW HEART STAY TRUE?

Developed by South San Francisco, Calif.-based XDx Inc., AlloMap Molecular Expression Testing is essentially a blood test that detects the absence of rejection in a transplanted heart. This real-time, quantitative PCR assay, performed in XDx's CLIA-certified reference laboratory, measures the expression levels of 20 genes associated with cardiac allograft rejection in a patient's peripheral blood and generates a score that detects the absence of rejection.

"Right now, the test is used by cardiologists who manage heart transplant recipients," explains Pierre Cassigneul, XDx chief executive officer. "Currently, the test is used to assist in monitoring patients

six months and beyond their transplant surgery."

Test scores can range from 0 to 40 — the higher the score, the greater the risk of rejection. To make certain that scores are reproducible, each time a blood test is done, each gene is tested in triplicate. The important measure, Cassigneul says, is the Negative Predictive Value (NPV). For example, in patients one year post-transplant or greater, a score of 34 or less has an NPV >99 percent. This means a patient in this group with a score of 34 or less has less than a 1 percent chance that they are rejecting the transplanted heart.

The major advantage of this genomic test, says Cassigneul, is that, in many cases, it can eliminate the need to do an endomyocardial biopsy. "We believe we are meeting a very important unmet medical need, in that cardiologists are dissatisfied with biopsies because they do not detect rejection until it is present." Biopsy results, Cassigneul says, are highly variable. "Sampling is done in a blind way, even though it's done with an imaging system and physicians take four to five samples at one time — it is still a sampling process, and, therefore, one might 'miss' the tissue indicating rejection." Additionally, biopsies create scar tissue, which can be confused easily with rejection. The real need, Cassigneul points out, is for an indicator that can detect early and without the associated confounders of biopsy.

Immunosuppression therapy with corticosteroids is a key component of post-transplant therapy, but not without some well-known side effects, notably osteoporosis and diabetes. "The reason our test

for the moment is limited to six months after transplantation," says Cassigneul, "is that most cardiologists will use high-dose steroids right after surgery to suppress the patient's immune system sufficiently to allow the body time to accommodate the new organ. What we have seen with the AlloMap test is that patients react very differently to steroids. We believe that once the Cardiac Allograft Rejection Gene expression Observational (CARGO) study [the first AlloMap clinical trial] is published, we will be able to demonstrate that the AlloMap test provides additional information to the cardiologist to indicate how a patient is responding to corticosteroids.

"These data are forthcoming," he continues, "and, therefore, use in the period from zero to six months post-transplant is not yet recommended."

An AlloMap test costs \$2,950, compared with a biopsy cost ranging from \$3,000 to \$5,000, and will most often be less expensive, Cassigneul says. Even when the test score is high and cardiologists say, "I'm not sure, I'm going to do a biopsy anyway," a complementary biopsy will still result in savings for the health care system about 20 percent of the time, he says. Private insurers already cover the test on a patient-by-patient basis, and Cassigneul believes publication of the CARGO study and greater clinical use will advance the coverage process for this test.

XDx also is developing other tests focused on immune-related diseases. One is a similar application for lung transplant rejection. Another, which particularly excites Cassigneul, will be for an auto-



PHOTOGRAPH BY HAROLD CLARK

**“To adequately screen** the U.S. population with colonoscopy, you would have to train thousands of additional gastroenterologists,” says ChondroGene’s K. Wayne Marshall, MD, PhD. “So, there’s a real, critical need to develop a broad, population-based screening tool.”

immune disease like lupus. “It will significantly expand an opportunity for us to address a critical need for patients and doctors. The disease in question is very hard to diagnose, but more importantly, it’s a disease that is not constant—it’s relapsing and remitting.”

From time to time, for reasons not clear, the disease flares and the damage to whatever it targets can be significant, Cassigneul explains. The only way to treat those patients is to put them on immunosuppressive drugs in the hope they will lower the impact of those flares. “It’s a difficult balance because immunosuppressants have strong side effects, such as enabling infections, long-term toxicity to kidneys and

liver, and skin cancer. It’s hard to balance the short-term benefit with the long-term risk. Clinicians prefer to give the drugs or intensify their use only when it’s necessary, either at the flare or just before. The AlloMap test, we believe, will help them to manage their patients better.”

#### **FROM BROAD STROKES TO SPECIFIC MARKERS**

ChondroGene, a Toronto-based company formed in 1998, teamed with the University of California—San Francisco and others to develop genomics-based screening tools for a number of disease states, primarily cancers. The company is developing a blood test, based on an approach the company calls the

Sentinel Principle, to identify biomarkers to screen for colorectal cancer and precancerous polyps. The test could be ready for use within the next two years.

“The concept underlying the Sentinel Principle was developed by C. C. Liew, PhD, our chief scientist, and is very simple,” says K. Wayne Marshall, MD, PhD, CEO. “As blood circulates in the body, it acts as a sentinel by reflecting and responding to external and internal environmental influences. The blood also facilitates cell-cell interactions and maintains homeostasis in the body as it passes through various microenvironments within the body.

Alterations in gene expression in the blood, Marshall believes, occur

as a result of interactions with these microenvironments. “These alterations result in disease-specific profiles, or molecular signatures, that typically span a few hundred genes, which we use as a ‘broad strokes’ readout as to what is happening in the body. We can then ‘mine’ for a smaller number of markers, and by multiplexing them, improve on their specificity and sensitivity.”

In what he calls “one of the twists we don’t expect to happen,” Marshall, an orthopedic surgeon doing primarily knee surgery, teamed up in 1998 with C. C. Liew, an expert in the cardiovascular genomics area then at the University of Toronto and later invited to Harvard University to found and direct the Cardiovascular Genome Unit at Brigham & Women’s Hospital. They came together to apply functional genomic techniques to the field of osteoarthritis, which led to the start of ChondroGene and resulted in a successful collaboration with Pfizer. Marshall and Liew soon realized that their Sentinel Principle approach, valuable as it is, would not be helpful in the early diagnosis of osteoarthritis because of the lack of disease-modifying drugs. But, Marshall says, they knew that diseases like cancer could often be cured if diagnosed early enough.

“The best way to cure cancer is not with new drugs, because by the time you are using drug therapy, you’ve got disease that has spread and you’re trying to prolong life. Most of the time, chemotherapy is not going to save lives. The best way to cure cancer is to make the diagnosis at a very early stage, ideally before symptoms occur, so that

treatment can be provided before the cancer has a chance to spread.”

Screening guidelines for colorectal cancer, which is highly preventable, call for annual screenings of adults over the age of 50. The problem with traditional tests in this area, Marshall says, is that the primary test, the fecal occult blood test (FOBT), has suffered from a lack of sensitivity and patient compliance because patients don’t want to deal with a stool-based test. Sigmoidoscopy, a much more sensi-

**“There is too little and too much colonoscopy performed. We need a test [to] identify patients at risk of colon cancer.”**

— K. WAYNE MARSHALL, MD, PHD,  
OF CHONDROGENE

tive test, is invasive, and while it can accurately visualize the lower half of the colon, it misses the other half. The gold standard for colon cancer screening, colonoscopy, can access the whole colon, but the procedure is invasive, costly, and not without possible complications.

“The reality is that colonoscopy is too expensive to be used as a broad population-based screening tool,” Marshall points out. “It is clear that there is a critical need to develop a test for colorectal cancer screening that is accurate, affordable, readily accessible, and minimally invasive. A test meeting these criteria would promote compliance by patients and likely be readily adopted by both physicians and payers.”

As ChondroGene moves toward clinical trials with its blood-based screening test, it expects that per-

formance of the test will guide how often it should optimally be given. Marshall thinks it likely will be every 3 to 5 years. “Only 40 percent of the population undergoes any kind of screening, and for the subset that does undergo colonoscopy, only about 10 percent have cancer or the types of polyps that have a high risk of becoming cancerous. The paradox is that there is both too little and too much colonoscopy being performed. To reduce the number of normal colonoscopies that are done and to target the individuals who need it the most, we need a test upstream of colonoscopy that can identify patients who are at a higher risk of having colon cancer or precancerous polyps. These patients could then be moved on to colonoscopy for cancer biopsy and follow-up surgery, or for

polyp excision to prevent subsequent development of cancer.”

Beta testing of a prototype test is expected to begin at several sites in the first half of 2006. Cost of the test, Marshall says, is expected to be less than a colonoscopy — which currently costs \$1,200 to \$1,500 — and more than a fecal occult test, which ranges from \$30 to \$50. Marshall recognizes the competing interests involved. “Physicians and patients want tests with high sensitivity and specificity that are easy and simple to use. However, tests must also be affordable for payers of healthcare, so it is important that any test have a reasonable price point.”

The tipping point is all about change that makes a difference. These tests and others to come offer new, and perhaps better, options for patient management. **BH**