

# MOLECULAR DIAGNOSTICS:

## PERSONALIZING PERSONALIZED MEDICINE

Thirteen years after Roche launched the industry with a test for HIV load based on transcript abundance, molecular diagnostics is in full flower. A \$3.3 billion market growing at 17 percent annually, the field includes assays for disease predisposition, screening, diagnosis, prognosis, monitoring, and predicting treatment efficacy, using markers ranging from SNPs to methylcytosine, messenger RNA to microRNA. Bringing digital power to traditional analog medicine, the field “is absolutely going to revolutionize health care,” says Harry Glorikian, managing partner at Scientia Advisors. “I believe it with every fiber in my being.”  
By Jeffrey M. Perkel

About a year ago, 48-year-old Jeff Gulcher’s doctor called to say Jeff had cancer. His prostate was teeming with cells scored at Gleason-6, the high end of intermediate grade. The recommendation: radical prostatectomy.

The diagnosis was shocking. Gulcher wasn’t at risk as far as he knew: he had no family history of early prostate cancer, and he had no symptoms. What he did have was a suspicious genotype.

Gulcher had paid \$985 for the deCODEme test, a genome-scale single nucleotide polymorphism screening service from deCODE Genetics. The test uses Illumina microarrays to profile about a million loci, reporting back on the relatively small number of conditions that can be tied to the results. Scattered across 10 chromosomes, the test’s 13 prostate cancer-related markers suggested that Gulcher’s lifetime risk for the disease is about twice the average, or 32 percent. That’s not a diagnosis, but a warning, like elevated cholesterol. It means, pay attention.

Gulcher’s physician recommended a prostate specific antigen (PSA) test. Such screening typically doesn’t begin until age 50. In this case, the test came back at 2.0 ng/mL, “in sort of the mid-normal range,” Gulcher says. Normally harmless, that value plus the genotype data prompted his urologist to refer Jeff for a biopsy.

“I’m thinking, there’s no way I have cancer,” Gulcher recalls. “I’ll go through the motions, have the biopsy. But just having this higher risk doesn’t mean I’m going to get prostate cancer by any stretch of the imagination.”

Fortunately, Gulcher didn’t rely on his intuition, and the surgeon excised his tumor intact; postoperatively, it was reassessed at high grade. PSA score down to zero, Gulcher appears to have dodged a bullet, all thanks to a molecular diagnostic that he actually helped develop. Gulcher is deCODE’s co-founder and chief scientific officer.

“This genetic test is reclassifying people who are thought to have average risk into somebody who really is at higher risk, and may benefit from extra surveillance,” Gulcher says.

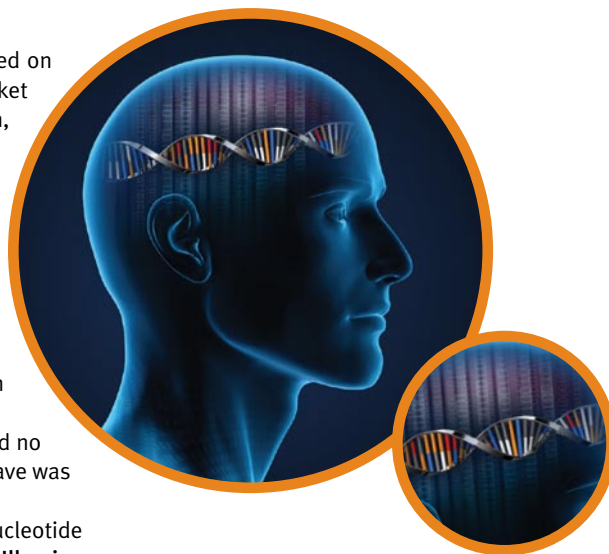
That, in a nutshell, is the promise of molecular diagnostics (MDx). Whether stratifying patients for heightened or diminished scrutiny, advising doctors on treatment decisions, or diagnosing disease, the field is redefining medicine.

### From Analog to Digital

Every diagnostic is molecular, whether measuring cholesterol for heart disease, radiolabeled glucose for brain imaging, or mass spectrometric peaks for ovarian cancer. So just what, exactly, is a “molecular” diagnostic?

According to Harry Glorikian of Scientia Advisors, the term generally applies to assays that detect nucleic acids, whether single nucleotide polymorphisms, mutations, or RNAs, with a sprinkling of “certain very high-quality, high-value protein assays,” as well.

They come in a variety of flavors. Besides predisposition tests like deCODEme, there are assays for diagnosis, prognosis, prediction of drug response, and disease [continued >](#)



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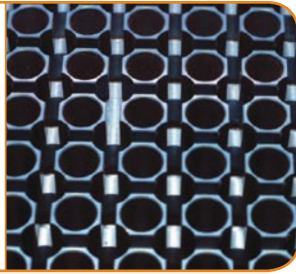
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## Molecular Diagnostics

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monitoring. Such tests, says Glorikian, represent a fundamental break from what he calls the traditional “gestalt” diagnostic approach. Blood pressure, temperature, and blood chemistry “are not digital indicators but analog indicators,” Glorikian says, “where [the doctor] uses the computer between his/her ears, looks at all this stuff, bins it together, and says aha! This is what I think.”

By contrast many MDx tests provide quantitized, digital data, as well as actionable results.

Take Down syndrome. Traditionally, a woman’s likelihood of having a baby with trisomy-21 is first assessed by noninvasive screens based on maternal serum proteins and ultrasound. Yet these assays provide risk scores rather than absolute diagnoses, and must often be supplemented with more accurate, but also invasive tests such as amniocentesis, which literally count fetal chromosomes.

Sequenom’s trisomy-21 MDx, set for launch later this year, blends these two approaches. Using mass spectrometry to count fetal chromosome 21–derived transcripts in maternal blood, the test is both noninvasive and quantitative—though not, per se, digital; the test measures the ratio of maternal and paternal alleles from chromosome 21.

“It’s an absolute result,” says Harry Stylli, **Sequenom’s** president and CEO. “You’re not dealing with a risk score.” As a result, high-risk pregnancies may be properly managed, while the number of pregnancies unnecessarily subjected to invasive procedures—currently about 140,000 per year, Stylli estimates—“will dramatically decrease.” [Editor’s Note: It came to our attention shortly after publication of this article that the release of Sequenom’s test has been delayed due to questions about some of their test data.]

### The Sequence Space

Perhaps no genes more starkly illustrate the actionable power of MDx than BRCA1 and BRCA2. Mutations in these genes carry such high risk of breast and ovarian cancer (about 82 percent) that some carriers opt for prophylactic radical mastectomy, using **Myriad Genetics’** *BRACAnalysis* blood test to help guide their decision.

Based on full-length Sanger dideoxy gene sequencing, *BRACAnalysis* is an unusual test; sequencing is more typically suited to research labs. Yet unlike proteins or transcripts, Gulcher notes, DNA “is the only stable molecule in your body.” It enables Myriad to pick up novel mutations. “Unfortunately there are a very large number of mutations in the genes that we look at, so it is not a mutation-specific test, it is a full DNA sequence test,” explains Pete Meldrum, Myriad’s president and CEO.

Myriad offers the test as a lab-developed “home brew” assay available through the company’s Clinical Laboratory Improvement Amendments (CLIA)–certified facility in Salt Lake City, Utah. Not yet vetted by the US Food and Drug Administration (FDA), such tests can only be offered by the lab that develops them.

In this case, results come back in about 10 days. That’s not generally a problem for predisposition tests. But sometimes, and especially

when confronting acute medical issues, patients don’t have that kind of time.

“In general, the faster the results become available, the more likely it is that they are going to be actionable,” says David Persing, executive vice president and chief medical and technology officer at Cepheid.

### Testing on Demand

When speed is required, real-time or quantitative PCR (qPCR) delivers. Most labs have a PCR machine, and most lab techs are competent to run them.

“Real-time PCR is quite a forgiving technique,” says Stephen Little, CEO of DxS. “You can put different levels and quantities of samples into the test and it will still give a good result.”

Even so, clinical labs often delay crucial tests because they must process them in batches. Cepheid’s GeneXpert system was built to circumvent this problem, says Persing. A modular, independently addressable real-time PCR system, the GeneXpert is “the first sample-in, answer-out machine for real-time PCR to hit the market,” says Glorikian.

Anyone can use it, Persing says. In one study, nurses running the company’s Group B *Streptococcus* test in the labor and delivery suite “delivered results that were highly accurate, and were as reliable and as good as the ones delivered by the lab.”

In part, that’s because there’s no sample prep; the system processes raw samples straight to analysis. Results can arrive in as little as 31 minutes, fast enough to catch patients before they leave the doctor’s office.

Cepheid’s new Xpert MTB/RIF assay reports in less than two hours both if an individual has tuberculosis and whether it is resistant to rifampicin. Traditional culture methods take weeks to make that assessment, says Persing, during which time patients may unwittingly spread the infection.

Cepheid’s assays are mostly based on **Applied Biosystems’** TaqMan reagents, modified oligonucleotides that contain a fluorescent dye on one end and a quencher on the other. During PCR, this oligo binds its target DNA between the two amplification primers, where it is chewed up by the polymerase’s 5’ to 3’ exonuclease activity, separating dye from quencher in a burst of fluorescence.

DxS uses an alternative detection reagent, called a “scorpion” probe, for its TheraScreen assays.

A scorpion, says Little, is “a self-quenching molecule.” A hairpin with a PCR primer at its 3’ end, the scorpion serves as a 5’ PCR primer. The hairpin is complementary to part of the amplified sequence, and like a TaqMan probe bears both a fluorophore and a quencher. In the absence of amplification, the closed hairpin keeps the two dyes in close proximity, dousing fluorescence. But after amplification, the probe can denature, unfold like a scorpion’s tail, and hybridize to the amplified segment. In the process, the fluorophore and quencher dissociate.

“That’s the signal that the reaction has taken place and we use it to signal the presence or absence of the mutations that we look for,” says Little.

In the case of DxS’s K-RAS and EGFR assays, the resulting data can guide treatment. Knowledge of a K-RAS mutation helps oncologists choose between cetuximab and panitumumab in colorectal cancer, while different mutations in EGFR affect which works better for nonsmall-cell lung cancer (NSCLC), gefitinib or erlotinib.

### Multivariate Assays

Though many MDx probe single nucleic acids, a small but growing number profile panels of genes simultaneously.

“As our understanding of genetics increases, we are going to learn that most diseases are multigenic in nature,” says Amit Kumar, president and CEO of **Combimatrix**, which develops MDx based on array comparative genomic hybridization; “It’s not just one gene that’s causing a particular illness or disease; it’s a combination of multiple genes functioning together.”

Three such tests have been cleared under the FDA’s “in vitro diagnostics–multivariate index assay” guidelines, all based on gene expression. **XDx’s** 20-gene AlloMap test uses reverse transcriptase (RT) PCR to monitor heart transplant rejection; **Agendia’s** 70-gene MammaPrint microarray assay predicts whether breast cancer patients are likely to relapse and benefit from chemotherapy; and **Pathwork Diagnostics’** Tissue of Origin test uses a 1,500-gene array to determine the source of a primary tumor in patients that present only with metastases.

“In order to effectively treat a metastasis, you have to treat it in the same way that you treat the primary tumor,” explains Ronen Tamir, chief commercialization officer at **Rosetta Genomics**, whose “miRview mets” test addresses the same problem using a 48-gene RT-PCR assay. In other words, a breast tumor that metastasizes to the colon should be treated as breast cancer.

Such tests may be less digital, perhaps, than the Down syndrome or BRCA tests—they sometimes provide probabilities rather than certainties—yet they still can help doctors make smarter treatment choices.

According to Steve Shak, chief medical officer at **Genomic Health**, whose 21-gene RT-PCR–based Oncotype DX assay also predicts breast cancer relapse, the most common treatment for women with node-negative, estrogen receptor–positive breast cancer is chemotherapy. Yet only about 4 percent of patients benefit from that option; the rest would do just as well with surgery plus hormonal therapy alone.

Now, using Oncotype DX, about one-third of treatment decisions change, Shak says. “Some women with high recurrence scores who might have been treated with hormonal therapy alone are then given hormonal therapy plus chemotherapy,” he says. Similarly, “there are many women who might have considered chemotherapy but based

on the results will be confident in getting hormonal therapy alone.” Agendia cites similar numbers for its MammaPrint diagnostic.

Yet when it comes to multivariate MDx, array-based assays like MammaPrint are relatively rare; frequently, developers use arrays for test development, reverting to RT-PCR once the hard work of biomarker identification is done. Such was the case for Oncotype DX and AlloMap.

“The thing with arrays is they are a little bit clunky to use, whereas RT-PCR, if you can use it, is a much easier technology to fit into a lab,” says Little. “Ease of use and convenience are very important considerations when you move into diagnostics.”

### Of Methyl-C and microRNAs

Ease of use and convenience are especially important when it comes to more esoteric markers, such as DNA methylation or microRNAs.

**LabCorp’s** ColoSure test measures methylation of the vimentin gene in stool samples. **Epigenomics** is developing a blood-based assay for *SEPT9* methylation. Both are early indicators of colon cancer, and both are based on qPCR detection of bisulfite-treated DNA (in which unmethylated cytosine is converted to uracil, while methyl-C remains unmodified).

“Having a very innovative test with a novel analyte [DNA methylation], we decided to be rather conservative when it comes to the assay technology,” says Achim Plum, senior vice president for corporate development at Epigenomics.

Slated for European release this year, Epigenomics’ assay won’t be the first blood test–based colon cancer screen on the market; **GeneNews** beat them to the punch in 2008 with ColonSentry, based on microRNA expression.

Short, endogenous, regulatory RNAs that silence the translation of complementary mRNAs, microRNAs are relatively recent discoveries. Yet they have emerged as hot commodities in MDx, and Cepheid, Rosetta Genomics, **Exiqon**, and Combimatrix are all also pursuing them.

Exiqon’s in-development assay for colorectal cancer prognosis is based on the detection of microRNAs with DNA analogs called Locked Nucleic Acids (LNA), because, says Søren Møller, Exiqon’s vice president of research and development, standard nucleic acids just aren’t specific enough.

“You don’t have much sequence space,” Møller explains. “microRNAs are 20–23 nucleotides long, and if you need to detect those in complex samples, you need very, very accurate detection techniques. LNA provides that advantage.”

Rosetta’s miRview squamous test uses RT-PCR of a single microRNA to help distinguish between squamous and nonsquamous NSCLC—an actionable result, says Tamir.

“Alimta is only labeled for nonsquamous NSCLC; Avastin is labeled for both,” he says. But a “black box” warning on Avastin “warns against fatal hemorrhaging of 40 percent of the patients which are squamous.”

A potentially life-saving decision based on a type of molecule researchers barely knew existed 10 years ago, it’s just another example of putting the “personal” in personalized medicine. The MDx landscape continues to change as new technologies—like next-generation sequencing—come to the fore.

“There’s a lot going on in this space, it’s so exciting,” says Glorikian. “Anybody who isn’t in this space needs to ask themselves why.”

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