

Cancer Biomarkers Expedite Detection

But Validation Must Be Improved to Obtain Truly Accurate Diagnostic Tools

K. John Morrow Jr., Ph.D.

It is widely acknowledged that early detection is the key to successful cancer treatment, yet such aggressive intervention requires accurate screening tools. Traditional diagnostic tests for cancer have a number of drawbacks including poor sensitivity, lack of specificity, and costly and invasive protocols. Now, finally, a variety of new biomarkers offers a wealth of possibilities. At CHI's recent "PepTalk 2008" meeting the results of studies on markers for lung, pancreatic, and various malignancies were scrutinized.

Telomerase

The need for early diagnosis is a powerful driver in the search for new cancer biomarkers. Edouard Nice, Ph.D., and his colleagues at the Ludwig Institute for Cancer Research (www.licr.org), recounted their efforts to develop early detection tools for malignancies. "Our strategy was to employ multidimensional, high-performance liquid chromatography to trace enrich low-level components such as growth factors in tumor material prior to the mass spectrometry analysis," he said.

Dr. Nice's studies have focused on a number of putative cancer biomarkers, but much of his discussion concerned telomerase, an enzyme long known to maintain the telomeres, or chromosomal ends, during cycles of cell replication. The decline of telomerase activity with age is thought to

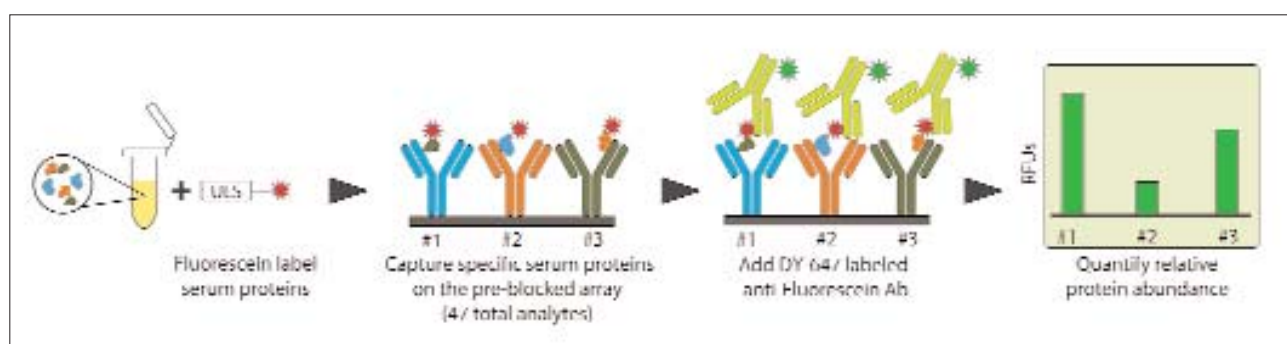
K. John Morrow Jr., Ph.D., is president of Newport Biotech Consultants and contributing editor for GEN. Web: www.newportbiotech.com. E-mail: kjohnmorrowjr@insightbb.com.

using a luminescent tag and then examined cells voided in the urine of bladder cancer patients and in the feces of colon cancer patients.

The gold standard for diagnosis and monitoring of bladder cancer has been cystoscopy combined with urinary cytology. This procedure has many drawbacks, including invasiveness, attendant morbidity,

autoantibodies in patients' serum, difficulty in automating cumbersome protocols, and cross reactivity of antibodies, all causing a surfeit of false positives.

With the need to assess specific proteins, a different set of issues present themselves, including the requirement for high sensitivity to analytes present in vanishingly small amounts, coupled with their miscreant



Gentel's Biomarker evaluation protocol using appropriate antibodies allows detection of multiple targets.

be responsible for chromosomal instability, resulting in aging and senescence.

There is a strong relationship between telomerase and tumor development; telomerase is expressed in more than 85% of cancers. In the Ludwig Institute's work, the enzyme is measured by using magnetic beads conjugated to the telomere recognition site, a group of repeated TTAGGG nucleotides. The cells are lysed and telomerase activity is detected by the incorporation of multiple biotinylated nucleotides into the growing DNA strands, which are then reacted with streptavidin-conjugated horseradish peroxidase. Dr. Nice and his colleagues validated this assay procedure

ty, and high cost. Since the vast majority of bladder cancers arise from the urothelium, analysis of the voided cells in the urine provides a potential source of cancer-specific markers.

The magnetic bead telomerase procedure is exquisitely sensitive, detecting as few as 10 cancer cells, and its accuracy was confirmed by an independent pathological review panel, according to Dr. Nice. Samples from normal healthy volunteers were all telomerase negative. In the future, the assay could be configured for high throughput and automation and is a prime candidate for a first-line assay for detection and monitoring of colon and bladder cancer, he added.

To this end, Dr. Nice and his colleagues are working with **Sienna Cancer Diagnostics** (www.siennadiagnostics.com.au) to move the technology forward. Sienna recently announced a licensing agreement with **Geron** (www.geron.com) for worldwide exclusive rights to develop telomerase for the in vitro diagnostic market.

Liquid Chromatography & Mass Spec

The combination of the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry is alive and well as a powerful diagnostic tool, reported Nigel Clarke, Ph.D., director of endocrinology at **Quest Diagnostics Nichols Institute** (www.nicholsinstitute.com).

Presently LC-MS is expanding beyond its traditional role at the front line of small molecule diagnostic measurements to large molecule (mainly protein) diagnostics. Previously, radioimmuno assay (RIA) for metabolites and hormone levels was the approach of choice, but it has too much baggage. Shortcomings to definitive RIA performance included the presence of

behavior. Dr. Clarke consigns to the bad-behavior category, molecules that are unstable, complex with other molecules, and clean up only with difficulty. Therefore, building new diagnostic tests requires robustness, small volumes, accuracy, precision, and reproducibility.

Dr. Clarke and his associates have studied the angiotensin system in the development of a superior LC/MS assay. Angiotensin II is the final active messenger of the renin-angiotensin pathway, it binds to a specific receptor, precipitating vasoconstriction and fluid retention, leading to an increase in blood pressure.

Since many drugs developed to control hypertension regulate blood pressure at various points in the renin-angiotensin system, it is critical to have accurate measures of the members of this cascade.

Dr. Clarke described his group's efforts to improve the preparative steps, automating them, and lowering the volumes of material required. The group found that LC/MS/MS assays required much less sample volume and were more specific than traditional RIA. One of the major problems of RIA that the group has resolved is its limited dynamic range, which extends from 0.65 ng/mL/hr to 14 ng/mL/hr. Dr. Clarke reported that his group's LC/MS/MS procedure improved sensitivity by an order of magnitude.

"We need to face new problems with protein analysis that do not exist in small-molecule assays," he said, "and external testing agencies need to be educated with regard to these new methodologies."

Ovarian Cancer Antigens

"There are several strategies for the early detection of cancer," says Michael Tainsky, Ph.D., professor at Wayne State University

See **Cancer Biomarkers** on page 28

News Genomics & Proteomics

Proteros and OSI Broaden Existing Protein Crystallography Collaboration

Proteros biostructures (www.proteros.com) and OSI Pharmaceuticals (www.osip.com) have expanded their protein crystallography partnership for the fifth time. Proteros will now also provide Gene-to-Structure services.

The extension follows the successful delivery of high-resolution, protein-ligand structures on multiple kinase targets under Proteros' Gallery Structure mode with compounds supplied by OSI.

The master service agreement, signed in October 2007, calls for Proteros to utilize its capabilities in construct design, protein expression and purification, as well as protein-structure determination to deliver a de novo protein structure and protein-ligand structures on a challenging kinase target.

Merck & Co. and Partners Identify a Gene-Expression Network that May Cause Obesity

Merck & Co. (www.merck.com) published two studies in *Nature* that provide evidence that genetic susceptibility to obesity involves changes in entire networks of genes and is not limited to mutations in specific genes.

In two related studies, Merck and collaborators report finding core groups of genes that have a causal relationship to disease traits, which include three previously unknown genes: *Lpl*, *Pmp11*, and *Lactb*.

The first study included Merck researchers and colleagues from the University of California at Los Angeles (www.ucla.edu). The second study involved the company, deCODE Genetics (www.decode.com), and the National University Hospital (www.landspitali.is). ■

Cancer Biomarkers Continued from page 26

School of Medicine (www.med.wayne.edu). "One can screen patients' serum for plasma DNA, antigenic proteins, marker proteins, or for antibodies."

This range of options opens up the possibility of developing a number of different detection protocols. It is unlikely that any single protein can serve as an unambiguous marker for malignancy, therefore effective detection systems will require that a number of different proteins be used. Thus, a definitive diagnosis would represent the composite value of several cancer-related markers.

Dr. Tainsky's program harnesses antibodies in patients' serum for the detection of cancer-specific epitopes using peptides selected for IgG binding from phage-display cDNA libraries. This detection

method is limited to amino acid epitopes and cannot detect epitopes produced through post-translational modifications such as those involving glycosylation or addition of lipid molecules to the original amino acid chain.

A prime target for a serum-based early detection test is ovarian cancer. Survival rates are quite high when the disease is confined to the ovary, yet early detection is challenging since symptoms are easily confused with many other illnesses. Often by the time the condition clearly manifests itself it is too late. Indeed, only 29% of cases are detected early enough to take remedial action.

Dr. Tainsky and his colleagues use microarrays to which phage displaying dif-



A researcher at **Sienna Cancer Diagnostics** performs an automated telomerase assay.



Gene Expression to Pathways

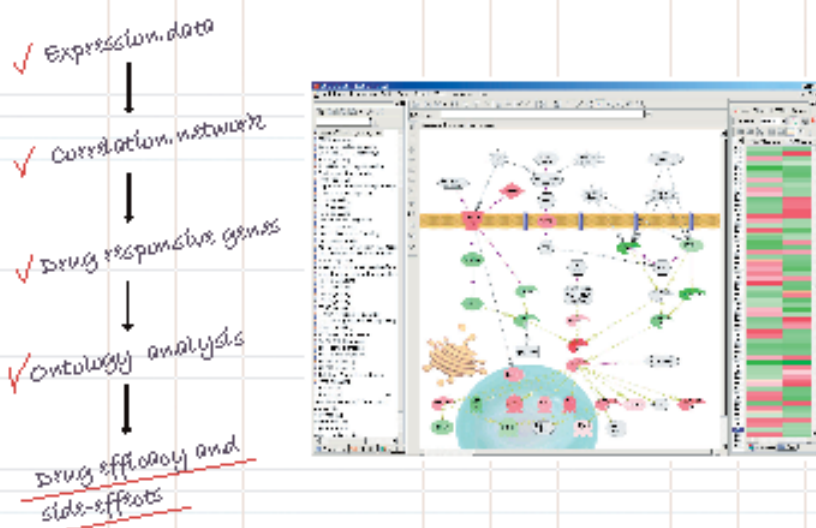
The Next Step



Pathway Studio®

From raw expression data to pathway model

Pathway Studio merges system biology data analysis with the richest-available source of pathways, protein functions and functional relations. Combining this complete data source with robust statistical approach of Gene Set Enrichment, Pathway Studio creates "The Next Step" in gene expression analysis, advancing research from profiles to pathways.



DOWNLOAD A FREE TRIAL AT www.pathwayanalysis.com

Ariadne Genomics, Inc. | 9430 Kay West Avenue | 240.453.5205 MAIN | info@riadnegenomics.com
Rockville, MD 20850 | 855.347.5040 TOLL FREE | www.riadnegenomics.com

ferent peptides from tumor cDNA libraries are bound. The phage are put through a number of rounds of selection and enrichment in order to produce highly tumor-specific clones, and the end products are robotically spotted to build the microarrays. Using this approach, the Tainsky team was able to isolate 65 antigen-producing phage clones positive for ovarian cancer samples, yet with low binding to sera from women with benign gynecological conditions.

The question of why these biomarker proteins stimulate an immune response in the patients goes to the origins of the cancerous state. According to Dr. Tainsky, the proteins are either normal proteins that are overexpressed so much so that the autoimmune-protective mechanisms of the individual break down, or they are part of the

normal repertoire, mutated to a state in which they appear foreign to the host.

The autoantibodies revealed through this technology can be used to detect ovarian cancer, and immunohistochemical studies have determined that some of the epitopes can serve as tissue biomarkers. They also offer the prospect of developing patient-specific cancer vaccines that could be individualized for the patient, Dr. Tainsky said.

High-Density Antibody Arrays

Designing a new generation of biomarker detection platforms requires reducing background to the lowest level possible. Bryce P. Nelson, Ph.D., research and development vp at **Gentel Biosciences** (www.gentelbio.com), discussed progress in building

See **Cancer Biomarkers** on page 30

Biomarker Technologies

Company	Program	Technology	Comments
Abbott Laboratories www.abbott.com	Biomarker research	PathVysion	DNA diagnostic for HER2 gene in breast cancer
Aperio Technologies www.aperio.com	Tissue biomarkers	Digitalizing slide-based data	Tissue-based biomarkers provide best indication of local tumor environment.
BioSystems International www.biosys-intl.com	Discovery & utilization of disease-specific protein biomarkers	mAb-based plasma screening	Designed to cut transition from biomarker discovery to clinical diagnostics
Caprion Proteomics www.caprion.com	Biomarker and target discovery collaborations	CellCarta	Targets for therapeutics development & identification of protein biomarkers is a multistep process that includes mass spec detection and quantitative peptide-expression profiling.
Expression Pathology www.expressionpathology.com	Protein biomarkers	Mass spectrometry	Biomarkers of lung cancer metastasis and survival
Genstruct www.genstruct.com	Identifying mechanistic biomarkers	Modeling powered by molecular profiling	Collaboration with Pfizer
GlaxoSmithKline www.gsk.com	Clinical trials for dementia	Translational clinical science	Pharmacology and discovery
Hoffmann-La Roche www.roche.com	Biomarker discovery	Systems biology approach	Biomarkers in clinical development and personalized healthcare for cancer drugs
Monogram Biosciences www.monogrambio.com	Theranostics Assays for R&D	Trofile HIV resistance tests; Veratag	For patient selection for maraviroc Optimization of background therapy; Platform to develop oncology test panels
Quest Diagnostics www.questdiagnostics.com	Angiotensin assays	LC/MS	Improvement over RIA
Schering-Plough www.sch-plough.com	Biomarker platform	Cancer biomarkers	Question-based pharmaceutical R&D process
Strategic Diagnostics www.sdix.com	Large collection of anticancer antibodies	DNA immunization	Constantly expanding catalogue
Vermillion www.vermillion.com	Ovarian cancer	Mass spectrometry	Profiles biomarkers in blood in preclinical stage

Cancer Biomarkers Continued from page 28

antibody arrays for research use.

In order to lower background, Gentel employs a nonporous, optically clear, ultra-thin nitrocellulose film that is attached to a 1 x 3 inch glass slide. The completed unit can be subdivided into 16 wells for multiplex assays containing more than 45 analytes per well.

The company has developed a single capture assay in which antibodies are bound to the surface. The sample to be tested is labeled with fluorescein and serum proteins are specifically captured. The sample is then reacted with a detector antibody (antifluorescein) labeled with a fluorescent dye, which can then be

measured using a standard fluorescence scanner.

The Gentel researchers identified candidate biomarkers through the literature and sourced high-performance antibodies, eliminating those that showed cross reactivity. The antibodies can then be quality controlled for reproducibility. Because of the small volumes required and the ability to subdivide the array into many individual sample wells, it is possible to generate a large body of data from a single slide. Many samples can be leveraged allowing the characterization of disease-related proteins and the response of regulatory proteins to therapeutic interventions.

Vermillion Reports Success of Ovarian Cancer Biomarker Panel

Vermillion (www.vermillion.com) says that use of its biomarker could help better identify women with ovarian cancer as well as improve early detection.

The first study used a model comprising the company's biomarker panel that was trained on a set of 270 samples from the University of Kentucky (www.uky.edu). This model, the ovarian tumor triage test, was then used in a prospectively collected cohort of 709 women from Rigshospitalet (www.rigshospitalet.dk).

The study provided validation that the test can distinguish malignant tumors from benign pelvic masses, according to the company. Results also showed that the biomarker panel could more than double the number of ovarian cancer cases referred to a gynecologic oncologist, thereby improving survival rates and reducing the number of surgeries performed, Vermillion reports.

The second study found that the panel of biomarkers in combination with CA-125 could more accurately identify early-stage ovarian cancer than CA-125 alone, the company adds. CA-125 is the only tumor marker for ovarian cancer currently available on the market. It, however, is not cleared for early-stage disease detection.

When examining stage-1 disease, the combination of the two markers correctly identified 87% of the cancers.

In the third study, Vermillion and the University of Kentucky Chandler Medical Center used SELDI TOF-MS to analyze ovarian cyst fluids and identify the underlying proteins that could serve as potentially useful biomarkers for ovarian cancer. They found that calgranulin A and B, two proteins commonly found in malignant ovarian cysts, may be useful in helping diagnose and predict prognosis of ovarian cancer. ■

Lung Cancer

Charles Birse, Ph.D., lung biomarker group leader at Celera (www.celera.com), reported that the currently available selection of lung cancer biomarkers is inadequate, represented by carcinoembryonic antigen, cytokeratin fragment 19, and neuron specific enolase. Not one of these markers provides the sensitivity and specificity required of a truly accurate diagnostic tool.

This shortage has spurred the company's search for better serum markers. The huge range of concentrations of different proteins in serum makes the search difficult and requires that the 97% most concentrated proteins such as albumin and immunoglobulin be removed prior to evaluation. But albumin is notorious for binding to other proteins, and its elimination may inadvertently remove possible biomarkers.

So Celera has an extensive program of discovery and validation, screening for shed proteins as well as cell-surface proteins in tissue specimens, cell lines, and serum samples. Using mass spec as a screening method, Celera researchers have identified over 500 possible disease-associated proteins. These investigations narrowed down the candidates that are expressed in the serum of patients, and finally yielded 27 targets as promising lung cancer biomarkers. The Celera researchers are also examining tissues, and Dr. Birse cautions that serum may not be appropriate for the validation of all biomarkers.

Pancreatic Cancer Biomarkers

Pancreatic cancer results in 30,000

deaths per year in the U.S., almost equal to the number of new cases. The five year survival rate is less than 5%, reported Ru Chen, Ph.D., a research scientist in the department of medicine at the University of Washington (uwmedicine.washington.edu). Early detection could significantly improve the prognosis of this deadly disease. When pancreatic cancer is detected in the early stage (when the tumor is smaller than 2 cm), the five-year survival rate is about 46%.

Dr. Chen's screening program is similar to many other cancer biomarker searches described in this article, including evaluation of pancreatic tissue, pancreatic secretions, and serum profiles from patients and matched controls. Proteins from normal and cancerous tissue are differentially labeled, broken into peptides, and affinity separated. This allows protein classification according to whether they are increased, decreased, or remain at the same level in the normal as compared to the malignant tissue.

Dr. Chen and her colleagues identified 500 pancreatic proteins that were unchanged, 95 that were upregulated, and 61 that were downregulated. They then designed a microarray platform in which paraffin specimens from a variety of normal and diseased tissue were mounted on an assembly that scanned them, using standard pathology staining, immunofluorescence staining, and immunohistochemistry.

Dr. Chen's group is now faced with a large collection of biomarker candidates. In order to prioritize them she has developed a list of criteria including degree of overexpression, role of the protein, availability of ELISA technology, whether the protein is extracellular or membrane bound, correlation with other analyses such as RNA and DNA detection, and the specificity of the protein as an authentic pancreatic cancer marker.

"Using this approach, we have identified several potential biomarker candidates that really work well on the cancer tissues. To be clinically useful for screening the general population, these tissue biomarkers need to be developed into blood assays and further validated in serum/plasma," Dr. Chen stated.

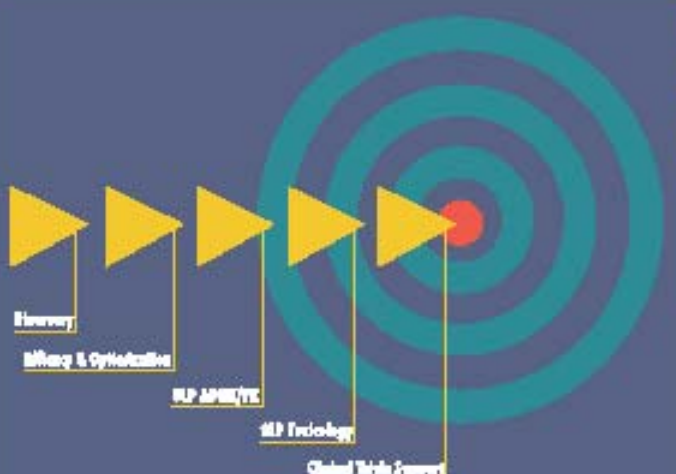
Discovery to Clinical Validation

There is an active field of cancer biomarker study that focuses on proteins that

See **Cancer Biomarkers** on page 32

SOUTHERN RESEARCH
 Legendary Discoveries. Leading Innovation.

**Industry-leading contract
 research in cancer and infectious
 disease therapeutics**



www.SouthernResearch.org
 (888)522-1166 | 001 (205)561-2890
BioDev@SouthernResearch.org
 See our website for career opportunities.

GEN Request Information

ADVERTISED PRODUCTS

Check out **Adlink** – GEN's online reader service. View ads online and contact the suppliers directly for more information about their company, products, and services.

[genengnews.com/
adlink.aspx](http://genengnews.com/adlink.aspx)

Cancer Biomarkers Continued from page 30

have been recognized as being associated with states of malignancy. Philip Hemkin, Ph.D., principle scientist, cancer diagnostics R&D, **Abbott Laboratories** (www.abbott.com), discussed his group's investigations of the tissue inhibitor of metalloproteinases-1 (TIMP-1).

Over the last decade a number of cancer researchers have observed elevated levels of TIMP-1 in the plasma of cancer patients, while healthy individuals maintain lower levels within a narrow range. These studies suggested to Dr. Hemkin and his colleagues that TIMP-1 might serve as an early detection signal for colon cancer.

The Abbott group developed an automated, two-step, double-mouse monoclonal sandwich assay in which an antibody against TIMP-1 is attached to a magnetic micropar-

ticle. A second antibody labeled with acridium will generate a light signal.

The group optimized the assay procedure, which has a large dynamic range, extending from zero to 2,500 ng/mL. The kit shows great precision and stability, he reported.

With a robust assay available, Dr. Hemkin and his colleagues are well positioned to continue obtaining a detailed clinical evaluation of the efficacy of TIMP-1 as an early marker for colon and other cancers.

DNA Immunization

DNA immunization, first introduced in 1999, is a technique for rapid generation of antibodies that until recently had not reached its full potential. **Strategic Diagnostics** (www.sdix.com) adopted this technology for

the purpose of producing large numbers of antibodies to putative cancer biomarkers. The approach involves the introduction of genes for target proteins (usually a sequence coding for a representative peptide of about 100 amino acids) into the animal to be used for the immunization procedure.

This results in a savings of time and resources since purification of a protein to a state satisfactory for use as an immunogen can be extremely challenging. With DNA immunization, a precise sequence is inserted into the carrier plasmid and there is no possibility of the inadvertent introduction of contaminating proteins.

According to Damon Hostin, marketing development manager, Strategic Diagnostics has optimized the procedure, overcoming problems such as poor immune response by the host animal that plagued the procedure in the past. "We broke the method up into its component parts, and then optimized the conditions of each step," said Hostin. "The algorithms for protein modeling have improved greatly in the last few years, so we are able to generate DNA sequences for peptides that are much better immunogens."

With present-day modeling, peptides can be produced that assume their appropriate 3-D folding, thereby ensuring that the authentic epitopes are available for stimulating the immune system of the host.

This means that antibodies can be produced on an assembly-line basis, avoiding the difficulty of expressing and purifying a protein that could be unstable and technically demanding. Over the course of its existence, the company has produced around 1,500 antibodies at its GMP facility, Hostin reported.

According to Leigh Anderson, Ph.D.,

CEO of the Plasma Proteome Institute (www.plasmaproteome.org), the search for cancer biomarkers is a harsh, unforgiving affair. "It is a high-risk enterprise, with a 99% attrition rate," he says. "This may be a reflection of the huge level of genetic variability within the human population."

What Works and What Doesn't

There is no guiding theory of biomarker science, Dr. Anderson noted, and it is completely empirically based. There is also a plethora of candidates that are difficult to resolve, and verification is slow, and appears to be a rate-limiting step. Despite the critical importance of cancer biomarkers in early detection, the protein-based in vitro diagnostics industry is only 1% as large as the pharmaceutical business. This may explain why, after years of investigation, the number of clinically successful biomarkers obtained through proteomics is zero.

"The major roadblock is the need for large (one to two thousand patients) sample sets when one is evaluating a candidate biomarker," Dr. Anderson said. "There are lots of possible biomarkers out there, but the amount of effort required for a really decisive assessment is immense."

Karin A. Hughes, Ph.D., worldwide product director for sepsis, stroke and protein C at **Biosite** (www.biosite.com), feels the problem may lie in the fact that discovery tools do not intersect with validation tools. "There are a large number of potential biomarkers but discovery technologies are not suitable for biomarker validation. Because the skills to validate them do not always reside in-house nor with discovery partners, biomarkers get stuck in the research phase and are not moving forward."

In order to move cancer treatment forward, an emphasis on diagnosis as opposed to therapy will be required. With therapeutic protocols running to hundreds of thousands of dollars per patient, effective biomarkers that detect cancer while it is still treatable look like a pretty good investment.

Dr. Anderson is optimistic that this day is dawning. "We have a project in place with the NCI to study 300 possible cancer biomarkers," he stated. "This will provide definitive information on the first large cohort of candidates." **GEN**