GEN genetic engineering news

Biotechnology from bench to business

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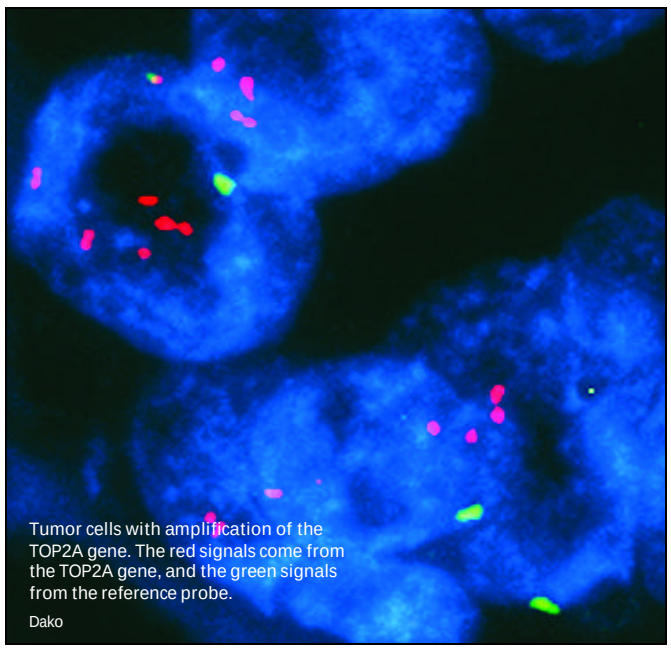
Diagnosis, Prognosis, and Therapy Selection



Tumor cells with amplification of the TOP2A gene. The red signals come from the TOP2A gene, and the green signals from the reference probe.

Dako

Molecular Diagnostics for Cancer Treatment Get the Right Drug to the Right Patient

Elizabeth Lipp

Methods of drug discovery and novel therapeutics have evolved greatly over the last decade, paralleled by advances in diagnostic tools. Although there are currently multiple methods of diagnosing and staging cancers based on tissue histology, only recently has the idea of molecular cancer diagnosis and profiling gained traction and acceptance. Presentations at the upcoming Cambridge Healthtech "Molecular Diagnostics Conference" in San Francisco will cover the latest in molecular diagnostics.

Stephen Little, CEO at **DxS** (www.dxsgenotyping.com), notes that acquired mutations in genes, such as *EFGR* and *K-RAS*, seem useful in diagnosis, prognosis, and therapy selection in cancer. "There is a trend in the industry to move away from cytotoxic drugs to targeted therapies," says Little. "And the need for tests to target these drugs correctly is the focus of what we do. Our products detect mutations in tumors

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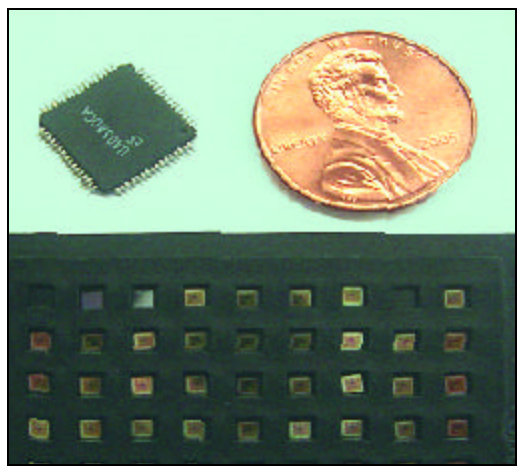
$1,000 Genome Remains the Holiest

Innovations and Advances Move Sequencing Forward, But the Ultimate Goal Will Continue to Be Elusive

Kate Marusina, Ph.D.

With the advent and development of high-throughput technologies, DNA sequencing takes center stage as a powerful tool in decoding genetic information. However, the goal of sequencing many genomes in parallel can only be made by considerable reduction in sequencing costs. A price tag of $1,000 per three gigabases—the size of the human genome—has become the theoretical benchmark.



A tray of unpackaged silicon integrated circuit chips containing the nanopore and high-speed sense electronics to detect nucleotide sequences of DNA (bottom). A packaged nanopore circuit chip in a familiar electronic quad flat pack (top)

Circuit design from David Kotecki and Chip fabrication courtesy of National Semiconductor

The current challenge is to reach a price-performance point that would enable previously impossible genome-wide studies. Making use of genomic information will require extensive validation of correlations between mutations and phenotype. This can be done either by large-scale association studies, bioinformatic predic-

See Whole Genome Sequencing on page 32

Sticky Ends

The **Oligo Factor** opened new oligo synthesis facility in Holliston, MA... **Symyx Technologies** released v. 4.0 of its Software Discovery Notebook... **Thermo Fisher Scientific** acquired **SwissAnalytic Group**, which owns **Spectronex** (mass spec and chromatography equipment) and **Flux Instruments** (HPLC pumps and software)... **Jivan Biologics** launched SpliceFold™ software package for analysis of alternative splicing from microarray data... **Affichem** appointed **SAFC Pharma** to provide chemical development services relating to Dendrogenine A, a potential cancer treatment... **Oxford Gene Technology** and **Agilent Technologies** inked deal giving OGT access to Agilent's microarray platform as an Agilent Certified Service Provider and naming Agilent as OEM supplier of OGT designed microarrays... **BioInvent** will collaborate with **Genentech** to co-develop and commercialize BioInvent's BI-204 antibody candidate for potential treatment of multiple cardiovascular conditions... **ChemAxon's** chemical visualization technology has been integrated with **Genedata's** lead discovery software solution... **BG Medicine** and **Applied Biosystems** entered into a collaboration agreement in which they will work

Cancer Diagnostics

Continued from page 1

to predict drug response."

Because mutations are localized to the tumor, there are specific technological challenges associated with their detection. "There is a need for diagnostic technologies with sufficient selectivity to allow the detection of mutations within a high background of unmutated DNA," says Little. "Real-time allele-specific PCR is a simple method that allows the detection of low numbers of mutant genes in high backgrounds of normal DNA.

"When you are looking at inherited SNPs, you either carry them in all of your cells or none. But tumors are different; when you're looking at cancer, you need to see the mutations against the background. This technology has been applied to several oncogene targets to develop selective and reliable diagnostic assays for *EGFR*, *RAS*, and *RAF* genes, as well as others.

"One emerging way to find these genes is in the plasma. When dealing with certain cancers, particularly cancer of the lung, biopsies are hard to obtain. It is well-known that the blood of cancer patients carries soluble DNA derived from the tumor so you can run one of these assays using DNA from blood, and that will help detect the mutation," says Little. "And hence determine the course that therapy should take."

Another application of the kits is monitoring the development of the tumor. As the tumor grows and develops, new mutations appear. Therefore, the idea of tracking the tumor and changing the treatment to vary with the appearance of these new mutations is taking hold. "Of course this approach only makes sense in the context of new therapies. What is particularly exciting at the moment is the combination of new therapies and new diagnostic tools that make this whole approach feasible," adds Little.

Rethinking Targeted Treatments

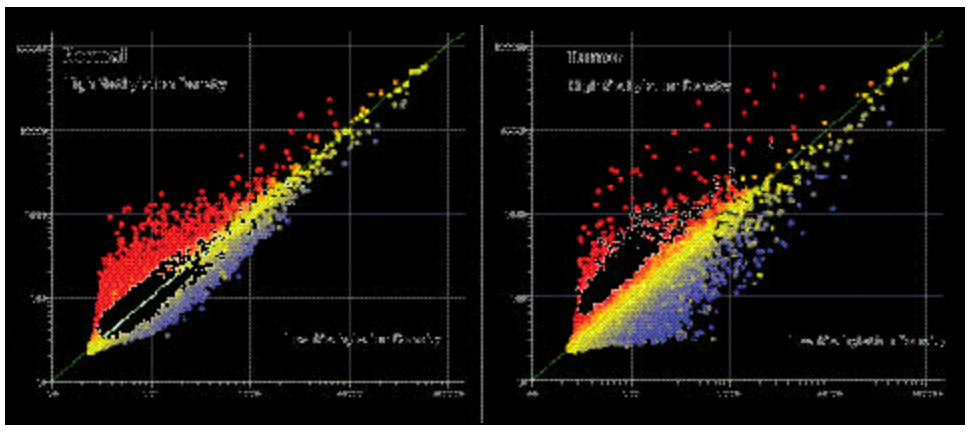
"We are standing at the crossroads and need to rethink how cancer is treated," says Jan Trost Jorgensen, principal scientist at **Dako** (www.dako.com). "The traditional methods of treating cancer are giving way to more targeted and personalized therapies and care, and here the predictive biomarkers will play an increasingly important role."

Dako's primary business area is cell-based cancer diagnostics with a focus on pathology. The pathology product area mainly comprises solutions for clinical use. The company focuses on the workflow in the pathology laboratories and provides system solutions for anatomic pathologists with an emphasis on cancer. This includes automated immunohistochemistry (IHC) systems, antibody- and molecular probe-based systems, and pharmacodiagnostics kits.

Dako has been a pioneer in the development of tests for targeted therapies and developed a predictive IHC *HER2* test for trastuzumab, which is used in the treatment of breast cancer. A number of anticancer drugs and diseases are now covered by new pharmacodiagnostic tests, such as *EGFR* inhibitors for colorectal cancer, anti-estrogens and aromatase inhibitors for breast cancer, and imatinib for gastrointestinal stromal tumors.

"Getting the right drug to the right patient is the goal of any medical treatment and is especially important for anticancer drugs due to their potential toxic effects and the seriousness of the disease," says Jorgensen. "During recent years, a number of slide-based biomarker assays with predictive potential have been developed. Based on a biopsy from the tumor it is possible with a certain probability to determine whether a patient is likely to respond to a specific anticancer treatment or not. These biomarker assays detect either if the tumor overexpresses a given protein, often the target that the anticancer drugs interact with, or if there are aberrations in the tumor DNA.

"The development of these pharmacodiagnostic assays should be seen as an integrated part of the drug development process as well as the targeted anticancer therapy itself. For new targeted therapies under development, an assay that is able to select the patient population that most likely will benefit from the drug needs to be developed in parallel," says Jorgensen.



Profiling DNA methylation patterns in cancer. The graphs show DNA methylation profiles of a tumor sample and non-tumor tissue-matched control sample. Profiles were generated by MethylScope® microarray-based technology from Orion Genomics. Each point on the scatter plot represents a methylation density measurement for a locus within the genome. Regions of high and low density DNA methylation measurements are labeled. The normal tissue DNA methylation profile (left) demonstrates the normal distribution of low to high density DNA methylation. A subset of loci that show differential DNA methylation density in the tumor relative to normal control sample DNA is highlighted in white. The DNA methylation profile of the tumor (right) demonstrates the global abnormalities in DNA methylation common in tumors, both gains (hypermethylation) and losses (hypomethylation) in DNA methylation density. The shift in the subset of data (white) in the direction of dense methylation indicates genomic loci that undergo hypermethylation in the tumor relative to normal cells. Identification of tumor-specific epigenetic changes may reveal DNA methylation-based biomarkers critical for disease diagnostics, prognostics, and therapy design.

Gene Expression-driven Diagnostics

Veridex (www.veridex.com), a **Johnson & Johnson** company, is developing molecular and cellular diagnostic assays for cancer. The CellSearch System is designed to automate and standardize the capture, enrichment, identification, and enumeration of rare circulating tumor cells (CTC) of epithelial origin in peripheral blood. The presence of CTC in the peripheral blood, as detected by the CellSearch Circulating Tumor Cell Kit, is associated with decreased progression-free survival and decreased overall survival in patients treated for metastatic breast cancer. A CTC count of 5 or more per 7.5 mL of blood is predictive of shorter progression-free survival and overall survival.

Using molecular technologies, Veridex is also developing innovative products to support the diagnosis, treatment, and survival of cancer patients. For clinicians, molecular tests offer the potential for greater accuracy and more patient-specific information than conventional tests. The company's first gene-based diagnostic test is designed to detect the spread of breast cancer to the lymph nodes.

Moreover, Veridex is developing multiple marker-based diagnostic tests to cover multiple pathways that lead to disease progression, focusing specifically on markers for breast, colorectal, and prostate cancer.

Molecular Diagnostics Market Assessment

Technological Innovation Continues to Refine these Effective Tools for Disease Management

Alfred R. Doig

In vitro molecular diagnostics have established themselves as effective tools for all aspects of disease management, especially in areas of unmet clinical need. Such tests have been developed for screening and prognosis as well as for applications, such as determination of genetic predisposition to disease, detection of presymptomatic disease, and prediction of individual drug response. Molecular diagnostics are the basis of pharmacogenomics, enabling the evolution of personalized medicine.

In Drug and Market Development's (D&MD) recently published market assessment report entitled "Molecular Diagnostics: Effective Tools for Disease Management", molecular diagnostics is taken to include tests for small-molecule, protein, or nucleic acid biomarkers and is mainly concerned with biomarkers that are measured using marketed clinical laboratory test kits or clinical analyzers. Biomarker assays are increasingly used throughout the process of disease management. A well-developed example is the use of HIV tests, including probe-based nucleic acid tests (NATs) for viral genotyping, in the management of AIDS patients.

Molecular diagnostic tests typically analyze key DNA, RNA, or protein biomarkers (analytes) to identify a disease, determine its course, evaluate response to therapy, or predict individual predisposition to a disease.

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Table 1. Market Growth of Immunoassay-based Molecular Diagnostics*

Application	2005	Percent	CAGR(%)	2010	Percent
General (hormones, metab, allergies)	6743.0	52.8	7.0	9458.0	48.4
Infectious disease**	4013.9	31.4	7.6	5789.4	29.6
Cancer**	1138.5	8.9	10.0	1833.6	9.4
Speciality** (mainly cardiovascular)	875.8	6.9	23.0	2465.6	12.6
Totals	**12771.7**	**100.0**	**N/A**	**19546.5**	**100.0**

* in thousands, ** includes proteomic assays

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"What we see right now is that cancer treatment is dispensed in a cookie-cutter manner," says Yixin Wang, group director, gene discovery and pharma biomarker support. "You have a patient who is in early-stage localized disease. How do you tell the difference between a patient who will go into remission and a patient who stands a greater chance of recurrence? This information will tell the physician whether or not the patient should receive more aggressive treatment, such as toxic chemotherapy.

"We have discovered gene signatures in breast and colon cancer that allow us to appropriately identify a high-risk patient," says Wang. "Now we are working on getting this tool to physicians to help them decide appropriate treatment."

Serum HER-2/neu as a Biomarker

Oncogene Science (www.oncogene.com), part of **Siemens,** has been doing work on the HER-2/neu oncoprotein, an important cellular target for the development of a variety of *HER-2*-targeted therapies since 1986. "This is going to be important for stratifying patients," says Walter Carney, head of Oncogene Science. "We have had the technology to measure circulating serum HER-2/neu since 1988, but it wasn't until Herceptin became available in 1998 that this way of doing science really seemed to move forward."

Serum HER-2/neu is different from traditional tumor markers such as CEA and CA-15-3. Serum HER-2/neu can help direct the use of anti-HER-2/neu therapies whereas traditional tumor markers are only useful for measuring tumor bulk. Serum HER-2/neu tells you not only about tumor load but also about the aggressiveness of the breast cancer.

The Oncogene Science HER-2/neu ELISA is a sandwich-type enzyme immunoassay that utilizes two monoclonal antibodies directed to the extracelluar domain (ECD) of HER-2/neu. According to Oncogene, independent studies demonstrated that this dual monoclonal antibody system shows no evidence of interference from Herceptin™ immunotherapy. The assay quantitates either the full-length molecule in tumor tissue (p185) or the ECD (p105) in serum, plasma, cell cultures, and fluids.

The capture antibody has been immobilized on the interior surface of microplate wells. To perform the assay, an appropriate volume of specimen is incubated in the coated well to allow binding of the antigen by the capture antibody. The immobilized antigen is then reacted with the detector antiserum. The amount of detector antibody bound to antigen is measured using a colored reaction product that is quantitated by spectrophotometry and reflects the amount of neu protein in the sample.

"Breast cancer is going to be the cancer that changes the way doctors perform medicine," says Carney. "It is one of the most treatable cancers at this point, and the awareness of it has made it possible for people to live longer and become better advocates for themselves. I am amazed at how many women act as their own doctor by telling their doctors what treatments are available and what course of action they want to take."

Carney points out that this awareness will spread to other kinds of cancer patients. "What we have been doing with serum HER-2 will eventually be used as part of a whole program that includes all three ways to test for cancer markers—tissue diagnostics, serum diagnostics, and imaging diagnostics. Eventually they will all come together to provide better patient management, leading to longer lives and a better quality of life for those living with cancer."

Mining the Cancer Epigenome

Jared Ordway, senior scientist and group leader of biomarker discovery at **Orion Genomics** (www.oriongenomics.com), says, "Tumor cells undergo epigenetic alternations including global changes in DNA methylation, a chemical signal that resides on the DNA and provides the cell with an adaptable mechanism for gene regulation. Unlike RNA, proteins, and other metabolic products, DNA methylation states are remarkably stable and therefore provide a robust biomarker platform with significant diagnostic potential."

To that end, Orion developed a technology called MethylScope to allow for accurate global profiling of DNA methylation density across the genome. Orion says a single MethylScope microarray is capable of quantitatively detecting the methylation status of each and every human gene. DNA from a tumor sample is labeled with dye probes that are able to distinguish between methylated and unmethylated DNA fragments. The fragments are hybridized to the MethylScope array and scanned, generating a methylation score for every gene on the array.

By comparing methylation profiles of two or more samples, Orion discovers biomarkers associated with specific diseases. MethylScope technology is capable of detecting inappropriate DNA methylation for all human genes on a single array, according to the company.

"MethylScope technology is uniquely suited to conduct genome-wide analysis for differential DNA methylation between clinical samples. This approach holds promise for identification of DNA methylation-based biomarkers with superior clinical sensitivity and specificity," claims Ordway.

Orion identified and validated a suite of novel breast cancer biomarkers, the most promising of which will be incorporated into the company's diagnostic assays for the early detection of breast cancer and the detection of breast cancer recurrence. Using MethylScope, they were able to quickly identify biomarkers in a panel consisting of normal and cancerous breast tissues. In a second independent biomarker validation panel of more than 200 normal and cancerous breast tissues, over 50 biomarkers demonstrated diagnostic potential, the most promising of which presented 90% sensitivity and 96% specificity. GEN