Volume 1, Number 1 • 02.15

Precision Medicine in Oncology®

The Journal of Precision Cancer Care

OncLive®

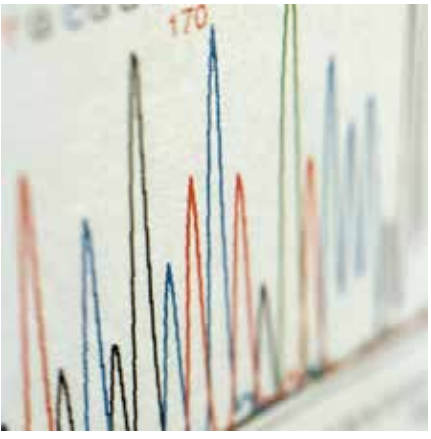
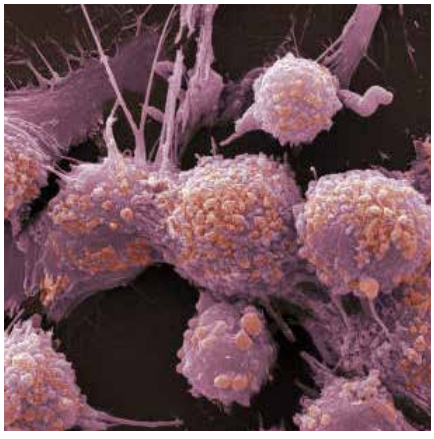
Dig Deeper Online at www.OncLive.com

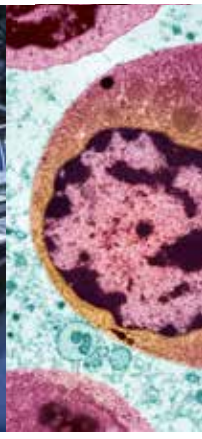
Precision Medicine in Oncology®

February 2015

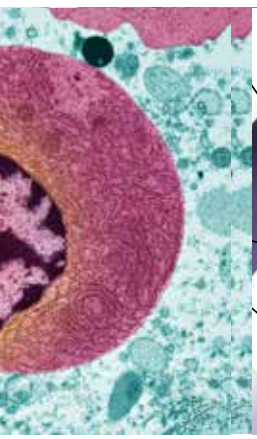
Volume 1, Number 1

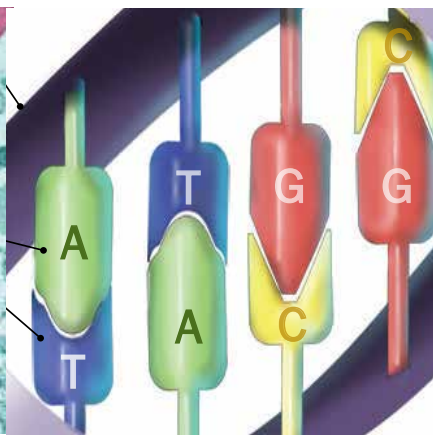
Table of Contents

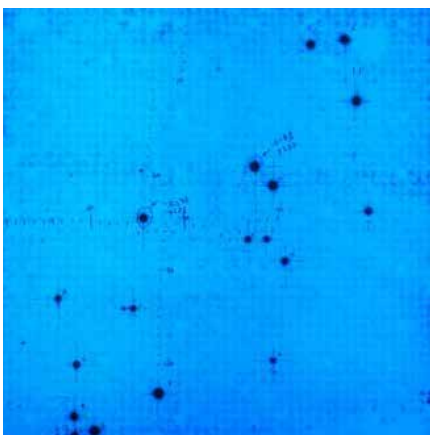





> As new research continues to reveal the biological drivers of cancer and costs fall for methods to detect ever more exquisitely defined subgroups of disease, tailoring medicine to the specific individual becomes less a goal and more a reality.







OncLive®

Intellisphere®
Oncology Specialty Group

Editorial & Production

Senior Vice President, Operations and Clinical Affairs
Jeff D. Prescott, PharmD, RPh

Senior Clinical Projects Manager
Ida Delmendo

Associate Editor
Jeanne Linke

Quality Assurance Editor
David Allikas

Art Director
Ray Pelesko

Senior Designer
Melissa Feinen

Sales & Marketing

Senior Vice President
Mike Hennessy, Jr

Vice President, Integrated Special Projects Group
David Lepping

Associate Publisher
Erik Lohrmann

Associate Director of Sales
Robert Goldsmith

National Accounts Manager
Albert Tierney

Digital Media Associate
Kristin Lopez

National Accounts Associate
Brittany Cain

Sales & Marketing Coordinator
Jessica Smith

Director, Strategic Alliance Program
Francine Durcan

National Accounts Manager, Strategic Alliance Partnership
Heather Shankman

Digital Media

Vice President, Digital Media
Jung Kim

Operations & Finance

Group Director, Circulation and Production
John Burke

Director of Operations
Thomas J. Kanzler

Controller
Jonathan Fisher, CPA

Assistant Controller
Leah Babitz, CPA

Accountant
Tejinder Gill

Corporate

Chairman and CEO
Mike Hennessy

Vice Chairman
Jack Lepping

President
Tighe Blazier

Chief Financial Officer
Neil Glasser, CPA/CFE

Executive Vice President and General Manager
John C. Maglione

Vice President, Human Resources
Rich Weisman

Vice President, Executive Creative Director
Jeff Brown

MH
Michael J. Hennessy Associates, Inc.

Office Center at Princeton Meadows
Bldg. 300 • Plainsboro, NJ 08536
(609) 716-7777

Looking Inside

How personalized medicine is changing diagnosis and treatments

Introduction

In the past decade, increased understanding of the biological underpinnings of cancer and improved technologies for detecting molecular differences between different tumors have created an opportunity for oncologists to more finely tailor diagnoses and treatments to individual patients. Known as precision medicine, much of the current research in this area is concerned with discovering and validating useful molecular biomarkers and developing reliable assays to detect them.

Types of Molecular Markers

A variety of different molecular markers are being investigated as potential biomarkers in oncology, including protein levels, gene mutations, and RNA signatures. Regardless of molecular type, these biomarkers can be classified in one of three broad categories depending on what clinical information they can provide: diagnostic, prognostic, or predictive.[1]

Diagnostic Markers

Diagnostic markers are primarily used to screen and detect cancers, especially those that present with few symptoms during early stages. These diagnostic markers can also help physicians to determine whether more risky and invasive biopsies are warranted in patients with symptoms suggestive of cancer. Early detection of such malignancies is often associated with improved patient outcomes and increased success of curative therapies.

A well-known example of a diagnostic cancer biomarker is prostate-specific antigen (PSA), which is discussed in more detail elsewhere in this supplement. Elevated blood levels of this protein are associated with increased risk of prostate cancer, a disease that is often asymptomatic at early stages.[2] Other FDA-approved diagnostic biomarkers include expression of c-KIT protein in tumor tissue, which can help to identify patients with gastrointestinal stromal tumors (GISTs), and the Risk of Ovarian Malignancy Algorithm (ROMA), which uses serum levels of human epididymis protein [4] (HE4) and cancer antigen 125 (CA125) to diagnose epithelial ovarian cancer in women with a suspicious pelvic mass.[3]

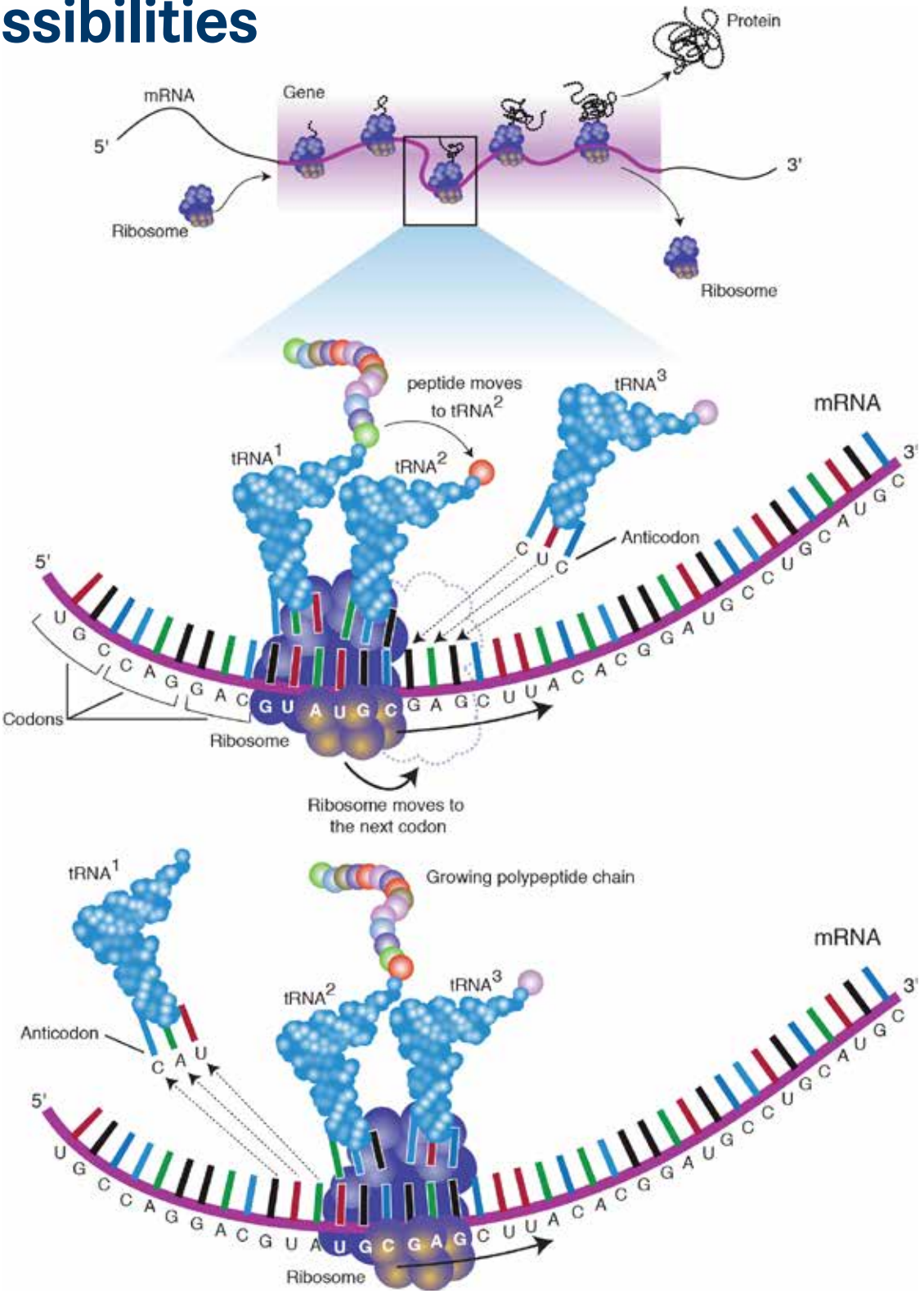
Prognostic Markers

Prognostic biomarkers are those that reveal information on the future course of a disease irrespective of treatment. In many cases, specific genetic mutations or abnormally expressed proteins are used to more precisely stratify cancers that arise from the same anatomical site but are driven by different oncogenic aberrations, and therefore have different growth characteristics.

The mutational status of the CCAAT/enhancer-binding protein alpha gene (*CEBPA*) is one example of a prognostic biomarker used in acute myelogenous leukemia (AML). Cases of AML that harbor mutations in both alleles of *CEBPA*, in the absence of other gene mutations associated with the disease, have a significantly higher 5-year survival rate than those with a mutation in only one or neither *CEBPA* allele.[4]

In addition to single-gene and single-protein biomarkers, gene expression profiles have been developed for use as prognostic indicators. The 70-gene Mammaprint® signature is an FDA-approved microarray assay used to assess the risk of disease recurrence in patients with breast cancer 5 years after diagnosis. Expanded immunohistochemistry (IHC) assays are also being developed that are based on the expression of multiple proteins in a tumor tissue sample. Assessing multiple molecular factors simultaneously allows a more complete profile of the disease, increased power to stratify patients, and reduced ambiguity in the results.[5]

Road Map of Possibilities



A genetic map is a type of chromosome map that shows the relative locations of genes and other important features. The map is based on the idea of linkage, which means that the closer two genes are to each other on the chromosome, the greater the probability that they will be inherited together. By following inheritance patterns, the relative locations of genes along the chromosome are established.

Predictive Markers

Biomarkers that can provide information on the likely outcome of a given treatment in a given patient are called predictive markers. In the context of targeted therapies, predictive markers frequently consist of an oncogenic mutation or the expression of the targeted protein in tumor cells. This is the case for the BCR-ABL fusion gene in Philadelphia chromosome–positive (Ph+) chronic myelogenous leukemia (CML) and the decision whether or not to treat with imatinib or other tyrosine kinase inhibitors (TKIs). The product of the fusion gene is the target of these drugs, and its presence can therefore predict a positive response to TKI treatment.

Some molecular markers can provide both prognostic and predictive information.

For example, the expression of human epidermal growth factor receptor-2 (HER2) in breast cancer cells is prognostic of shorter overall and disease-free survival, but it is also predictive of a patient's responsiveness to the cytotoxic drug doxorubicin.[6]

In addition to information about treatment responsiveness, predictive molecular markers can also be used to anticipate which patients may develop toxicities due to a particular treatment. The common chemotherapeutic agent 5-fluorouracil (5-FU), for example, is metabolized in the body by the enzyme dihydropyrimidine dehydrogenase (DPD). Patients with deficient DPD activity experience a high rate of 5-FU toxicity, including death.[7] Genetic analysis of the DPD gene prior to treatment can allow the patient and healthcare provider to prepare in advance for possible adverse effects (AEs), or avoid a particular treatment altogether.

Companion Diagnostics

When a test for a predictive molecular marker is paired with a particular treatment, it is known as a companion diagnostic. At a time when the role of targeted therapies is increasing in the field of oncology, the use of companion diagnostics is of crucial importance.

The efficacy of new targeted drugs for non-small-cell lung cancer (NSCLC), for example, depends on the presence of driver mutations that make tumors susceptible to the treatments. The TKI crizotinib has demonstrated efficacy only for patients with NSCLC that are positive for ALK fusion genes, but there are multiple different methods available for detecting such mutations, including fluorescence in situ hybridization (FISH), reverse-transcriptase polymerase chain reaction (RT-PCR), and IHC. Each of these techniques has advantages and limitations in terms of cost, complexity, sensitivity, and reliability,[8] and thus the FDA has approved a specific companion diagnostic, the Vysis ALK Break Apart FISH Probe Kit (Abbott Molecular Inc), for the purpose of detecting ALK rearrangements.

The consequences of molecular marker testing are considerable. Administration of unnecessary treatment can be costly and expose the patient to harmful AEs, while mistakenly withholding effective treatment can potentially be fatal. By using standardized, FDA-approved companion diagnostic tests, physicians and patients can be confident in making the correct treatment decisions.

Curbing Risk in Medical Innovation Through Personalized Medicine

The expanding applications of precision medicine in oncology rely on a concomitant expansion in the number of molecular tests administered to patients. This increased reliance on new tests of varying technical complexity has raised several issues, including the reliability and accuracy of molecular tests in large patient populations and the policy framework used to regulate such tests.

LDTs

Companion diagnostics for targeted therapies that are developed and manufactured by pharmaceutical companies are scrutinized by the FDA for safety and efficacy in a manner similar to drugs. However, many tests for molecular biomarkers are so-called "laboratory-developed tests" (LDTs), which are designed, manufactured, and used within a single laboratory. Historically, LDTs were simple to administer, available only to small numbers of patients, and applied to rare conditions.

FDA Proposals

In July 2014, the FDA announced it was planning two new actions to ensure the safety and utility of diag-

nostic tests. First, the agency will now require that companion diagnostic tests be submitted and approved in tandem with submissions for new targeted therapies. Because most targeted drugs are effective only in a specific subpopulation of patients, this requirement is intended to ensure that the necessary development of tests to identify such patients is not delayed, but rather undertaken as a part of the drug development pipeline.

The second step taken by the FDA involved the publication of a proposed risk-based oversight framework for LDTs.[9] This framework would phase in regulatory oversight, beginning with the highest-risk tests, including predictive diagnostics for cancer treatments. The proposal would involve enforcement of premarket review, quality systems, and AE reporting requirements. The stakes of safe and accurate testing are higher than previously thought because more people are being tested and the consequences of not receiving an effective therapy, or receiving an inappropriate treatment, could be serious. This is the rationale used for implementing the changes based on the level of risk involved with any given test.[10]

It is hoped that through these actions, the FDA will be able to both encourage the timely development of necessary companion diagnostics for new targeted agents, and also ensure that LDTs being used to inform crucial treatment decisions are both safe and accurate. At the same time, the FDA has vowed to continue its policy of enforcing discretion for LDTs for use with rare diseases and unmet needs in order to not stifle innovation in these crucial areas.

Hereditary Cancers: Which Patients Should Be Genetically Screened?

All cancers are associated with genetic aberrations that contribute to abnormal growth and other pathologies, but most of these are somatic mutations, which are acquired during a patient's lifetime, exist only in some cells, and are not passed on to offspring. About 5% to 10% of all cancers, however, are caused by inherited mutations that are transmitted from generation to generation and have the potential to affect multiple members of the same family.[11]

Cancer Syndromes

Causative mutations have been identified for over 50 hereditary cancer syndromes, which can be used to screen individuals to ascertain their risk of developing an inherited form of cancer. Many of these syndromes are associated with multiple different types of tumors, and may therefore present in different forms in different patients.

In some syndromes, the relationship between the disease and the causative gene or genes is relatively well defined. Familial adenomatous polyposis (FAP), for example, is caused by mutations to the adenomatous polyposis coli gene (APC). Mutations in APC act as a dominant autosomal trait, resulting in the formation of hundreds of colorectal adenomas that, if not treated, nearly always progress to colorectal cancer (CRC).[12]

Hereditary breast cancer and ovarian cancer syndrome (HBOC), in contrast, does not have such a straightforward etiology, despite the fact that it is one of the most common and well-studied inherited cancer syndromes. HBOC is also inherited in an autosomal dominant fashion, and is most often due to mutations in the *BRCA1* and *BRCA2* genes. These mutations, however, account for only 65% of the observed incidence of HBOC, while the remaining cases of the syndrome are caused by mutations in other DNA mismatch repair genes (MMR), genes associated with Lynch syndrome (hereditary nonpolyposis colorectal cancer), or as-yet-unidentified heritable factors.[13]

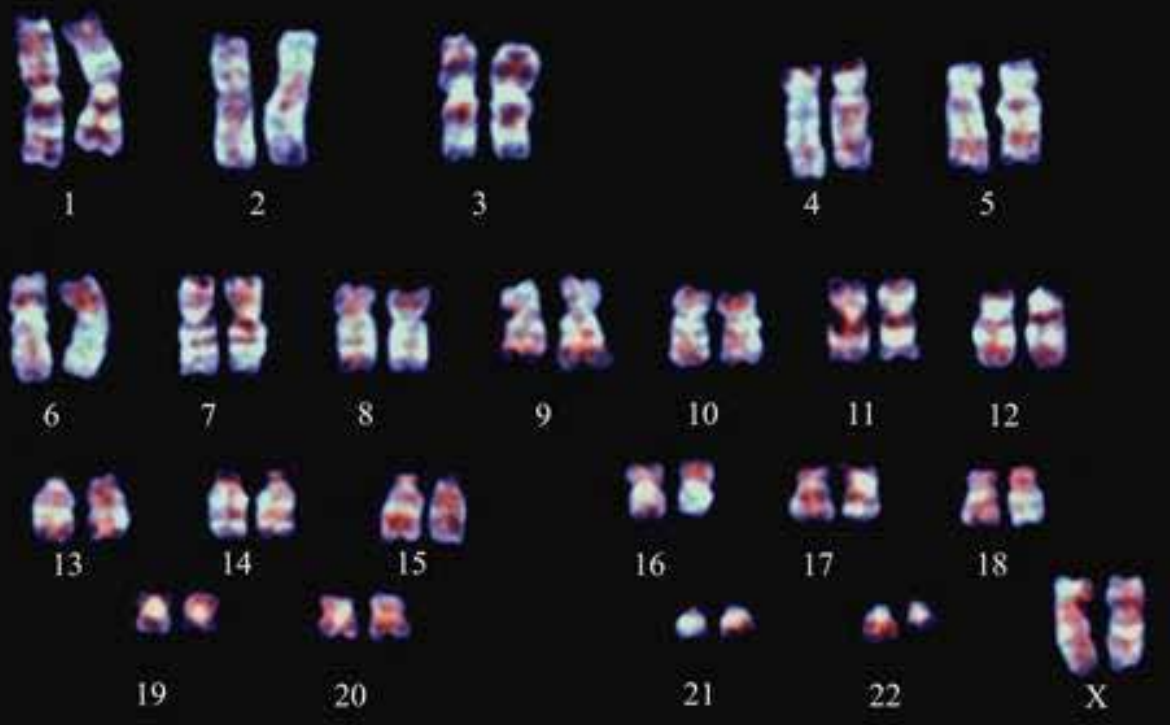
In addition to potential ambiguity in the origin of some hereditary cancers, many of these syndromes are also associated with the development of different types of cancers in different patients. Lynch syndrome is caused by mutations in one of several MMR genes and is inherited in an autosomal dominant manner. This syndrome increases an individual's predisposition to a wide array of cancers, the most common of which are CRC, endometrial, or ovarian cancer, but also include renal pelvis, pancreatic, small intestine, liver and biliary tract, stomach, brain, and breast cancers.[14]

Another important consideration for hereditary cancer syndromes is the fact that the causative genetic mutations have varying degrees of penetrance, and the identification of a mutation can only provide information on the risk of developing a given cancer. The benefit of this information should be weighed against the potential for psychological distress frequently experienced by patients who learn they are at increased risk for a potentially fatal disease.[15] Limited resources and potential anxiety incurred by unnecessary testing have placed practical limits on the scope of genetic testing for hereditary cancers; therefore, parameters to determine which patients should be tested must be addressed.

Practical Considerations

Because most cancer syndromes are relatively rare,

Philadelphia Story



Philadelphia chromosome. Colored light micrograph of a female karyotype (chromosome set) with one defective chromosome each from pairs 9 and 22. The defects, on the right-hand chromosomes of the two pairs, cause chronic myelogenous leukemia (CML). The 46 chromosomes of a human karyotype are here arranged in 23 pairs of a maternal and paternal chromosome. The left-hand chromosomes of pairs 22 and 9 are normal, whereas an exchange of material (translocational defect) made defective chromosome 9 larger and shrunk defective chromosome 22. In a bone marrow stem cell, the chromosome 22 defect causes the CML increased white blood cell count.

making the determination about which patients to refer for genetic testing can be difficult for nonspecialists, particularly in the context of limited patient awareness of their family history of cancer. Furthermore, many oncologists feel unqualified to discuss genetic test results with their patients.16 In order to assist general practitioners with these decisions, the American College of Medical Genetics and Genomics, the National Society of Genetic Counselors,[17] and the Commission on Cancer[18] have published guidelines for referring patients for hereditary cancer testing.

In general, the first step in determining whether an individual should be referred for genetic screening is a personal medical and family history. The personal medical history should include a personal history of benign and malignant tumors, illnesses, surgeries, biopsies and findings, reproductive history and contraceptive/fertility drug usage, and racial/ethnic background. The family history should identify which family members received cancer diagnoses, as well as items such as age at diagnosis, site of cancer origin, treatment received, and genetic testing results, if administered.[19]

Although personal and family medical histories might reveal multiple instances of cancer occurring within a single family, this is frequently due to chance or nonheritable risk factors, such as smoking, that do not warrant follow-up genetic screening. Some "red flags" revealed in family histories that would be suggestive of a heritable syndrome include cancers occurring at unusually young ages, more than one instance of childhood cancer affect-

ing a set of siblings, multiple types of cancer affecting one family member, multiple instances of a rare cancer type in a family, cancers affecting both of a pair of organs in a family member, and unusual presentation of cancer (eg, breast cancer in a male relative).[20] Race and ethnicity may also be a consideration for certain types of cancer, such as prostate cancer in African-American men or breast cancer in Ashkenazi Jewish women.

Risk Assessment Tools

There are multiple validated risk assessment models available to assist physicians in making an estimate of the likelihood that a patient is carrying a hereditary cancer gene mutation. Such an assessment can be valuable in helping to determine whether or not a patient should receive genetic testing, although some familiarity of the syndrome being considered, as well as full pedigrees spanning multiple generations, are needed to ensure that valid results are returned. Additionally, the probability calculated will vary depending on the specific model used, and should be considered when interpreting the results.[21]

There are many choices of risk assessment tools available for HBOC, including BRCAPRO offered by the University of Texas Southwestern Medical Center and BODICEA from the University of Cambridge, UK.[19] Other tools have been developed for non-BRCA hereditary cancer syndromes as well, including PREMM1,2,6 model for Lynch syndrome[22] and PancPRO for hereditary pancreatic cancer,[23] which are available from the Dana-Farber Cancer Institute website (http://premm.dfci.harvard.edu/, http://bcb.dfci.harvard.edu/bayesmendel/pancpro.php).

Direct-to-Consumer Testing

A relatively new development in the field of cancer genetics has been the creation and marketing of an ever-growing number of direct-to-consumer (DTC) genetic tests. For this type of testing, an individual sends a tissue sample collected at home to the company via postal or other delivery service. The results are then delivered to the customer online, by mail, or by telephone. Such services have raised serious ethical and practical concerns among healthcare providers and regulators.

One common concern about DTC testing is that rather than testing for mutations in known cancer susceptibility genes with direct linkages to hereditary cancer syndromes (eg, *BRCA1*, *BRCA2*), these tests identify common DNA variants that have a small impact on an individual's predisposition for cancer. Additionally, because these tests are primarily undertaken without the involvement of a physician or genetic counselor, important risk factors that may be discovered by collecting a medical and family history are not taken into consideration in formulating the final results. This has caused worry that consumers may be receiving a distorted, and possibly meaningless, view of their cancer risk.[24]

Since DTC genetic testing is most often conducted without the involvement of a physician or genetic counselor—either before or after the testing—there are also concerns regarding whether the information provided by these services is properly understood by consumers, and whether it might be causing actual harm. DTC genomic profiling frequently uses multiple low-risk alleles to determine cancer risks, but this type of complex genetic information is often misunderstood by the general public.[25] In addition, there is no opportunity in such a setting for a genetic counselor or other professional to address the potentially increased anxiety and other psychological harms that are often experienced when patients learn they are at increased risk for a possibly fatal disease.

The regulatory environment for DTC testing is currently unsettled. To this point, the FDA does not have a specific regulatory framework in place to address these products, although it does still exercise some control in this area. In 2013, the FDA ordered the genetic testing company 23andMe to discontinue providing information to customers regarding health risks for specific diseases. The agency took this action due to concern that such results were not clinically validated and were potentially misleading, although the company still provides ancestry analysis and raw genetic data.[26] Since then, because of the significant drop in new customers, 23andMe has been in constant communication with the FDA regarding a new submission that would meet with its approval.[27] However, many other DTC options exist for interested consumers, and debate continues over the proper role for these tests.

Conclusion

As new research continues to reveal the biological drivers of cancer and costs fall for methods to detect ever more exquisitely defined subgroups of disease, tailoring medicine to the specific individual becomes less a goal and more a reality. The array of molecular markers and genetic mutations that can help to determine the diagnosis and treatment of cancers is growing large enough that consideration must now be made as to how best to utilize these new tools. Not all biomarkers are created equal, and not all genetic tests are informative. Determining which provide the greatest amount of benefit for patient treatment is the next step toward precision medicine.

REFERENCES

1. Aftimos PG, Barthelemy P, Awada A. Molecular biology in medical oncology: diagnosis, prognosis, and precision medicine. Discov Med. 2014;17(92):81-91.
2. Thompson IM, Pauler DK, Goodman PJ, et al. Prevalence of prostate cancer among men with a prostate-specific antigen level < or =4.0 ng per milliliter. N Engl J Med. 2004;350(22):2239-2246.
3. Füzéry AK, Levin J, Chan MM, Chan DW. Translation of proteomic biomarkers into FDA approved cancer diagnostics: issues and challenges. Clin Proteomics. 2013;10(1):13.
4. Hatzimichael E, Georgiou G, Benetatos L, Briasoulis E. Gene mutations and molecularly targeted therapies in acute myeloid leukemia. Am J Blood Res. 2013;3(1):29-51.
5. Ward S, Scope A, Rafia R, et al. Gene expression profiling and expanded immunohistochemistry tests to guide the use of adjuvant chemotherapy in breast cancer management: a systematic review and cost-effectiveness analysis. Health Technol Assess. 2013;17(44):1-302.
6. Lee SB, Lee JW, Yu JH, et al. Preoperative serum HER2 extracellular domain levels in primary invasive breast cancer. BMC Cancer. 2014;14:929.
7. Nalejska E, Mączyńska E, Lewandowska MA. Prognostic and predictive biomarkers: tools in personalized oncology. Mol Diagn Ther. 2014;18(3):273-284.
8. Solomon B, Wilner KD, Shaw AT. Current status of targeted therapy for anaplastic lymphoma kinase-rearranged non-small cell lung cancer. Clin Pharmacol Ther. 2014;95(1):15-23.
9. Food and Drug Administration. Draft guidance for industry, good and drug administration staff, and clinical laboratories: framework for regulatory oversight of laboratory developed tests (LDTs). FDA website. 2014. http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM416685.pdf. Accessed January 28, 2015.
10. Shuren J. Curbing risk, not medical innovation, in personalized medicine. FDA Voice website. 2014. http://blogs.fda.gov/fdavoice/index.php/2014/07/curbing-risk-not-medical-innovation-in-personalized-medicine/. Accessed January 28, 2015.
11. National Cancer Institute. Genetic testing for hereditary cancer syndromes. 2013. http://www.cancer.gov/cancertopics/factsheet/Risk/genetic-testing Accessed January 28, 2015.
12. Vasen HF, Möslein G, Alonso A, et al. Guidelines for the clinical management of familial adenomatous polyposis (FAP). Gut. 2008;57(5):704-713.
13. Minion LE, Dolinsky JS, Chase DM, et al. Hereditary predisposition to ovarian cancer, looking beyond BRCA1/BRCA2 [published online ahead of print January 23, 2015]. Gynecol Oncol. pii: S0090-8258(15)00541-7. doi: 10.1016/j.ygyno.2015.01.537.
14. Abdel-Rahman WM, Peltomäki P. Lynch syndrome and related familial colorectal cancers. Crit Rev Oncog. 2008;14(1):1-22
15. Richmond-Rakerd LS. Modern advances in genetic testing: ethical challenges and training implications for current and future psychologists. Ethics Behav. 2013;23(1):31-43.
16. Hede K. Genomic testing: a struggle for oncologists. J Natl Cancer Inst. 2014;106(6):dju172.
17. Hampel H, Bennett RL, Buchanan A, et al. A practice guideline from the American College of Medical Genetics and Genomics and the National Society of Genetic Counselors: referral indications for cancer predisposition assessment. Genet Med. 2015;17(1):70-87.
18. Commission on Cancer. Cancer Program Standards 2012: ensuring patient-centered care.V1.2.1. 2012. https://www.facs.org/quality-programs/cancer/coc/standards. Accessed January 28, 2015.
19. Marie Lewis K. Identifying hereditary cancer: Genetic counseling and cancer risk assessment. Curr Probl Cancer. 2014;38(6):216-225.
20. American Cancer Society. Family cancer syndromes. 2014. http://www.cancer.org/cancer/cancer-causes/geneticsandcancer/heredity-and-cancer. Accessed January 28, 2015.
21. Antoniou AC, Easton DF. Risk prediction models for familial breast cancer. Future Oncol. 2006;2(2):257-274.
22. Kastrinos F, Steyerberg EW, Mercado R, et al. The PREMM(1,2,6) model predicts risk of MLH1, MSH2, and MSH6 germline mutations based on cancer history. Gastroenterology. 2011;140(1):73-81.
23. Wang W, Chen S, Brune KA, et al. PancPRO: risk assessment for individuals with a family history of pancreatic cancer. J Clin Oncol. 2007;25(11):1417-1422.
24. McCartney M. Margaret McCartney. Direct to consumer genetic testing–is all knowledge power? BMJ. 2015;350:h439.
25. Bellcross CA, Page PZ, Meaney-Delman D. Direct-to-consumer personal genome testing and cancer risk prediction. Cancer J. 2012;18(4):293-302.
26. Annas GJ, Elias S. 23andMe and the FDA [published online ahead of print March 13, 2014]. N Engl J Med. 2014;370(11):985-988. DOI: 10.1056/NEJMp1316367.
27. Seven months after FDA slapdown, 23andMe returns with new health report submission. http://www.forbes.com/sites/roberthof/2014/06/20/seven-months-after-fda-slapdown-23andme-returns-with-new-health-report-submission/. June 20, 2014. Accessed February 1, 2015.

Molecular Profiling Provides Treatment Clues Across Cancer Types

The treatment of patients with cancer has undergone a dramatic evolution in the past decade. Tumors are no longer classified based solely on histology. Molecular information has become a key factor for treatment selection, with a single mutation often impacting the entire course of therapy. The discovery of HER2 in breast cancer, EGFR in lung cancer, and BCR-ABL in chronic myelogenous leukemia set the stage for a genomic revolution.

Mapping the Breast Cancer Genome

Advances in the treatment and detection of breast cancer have led to a steady decline in breast cancer deaths in the last 25 years.1 Despite this decline, breast cancer is still the number one cause of cancer death in women, comprising 14% of cancer deaths in 2008.[2] Breast cancer also remains the second most common cancer diagnosis in women, with 23% of the total cancer diagnoses.[1,2] Genomic research has yielded a wealth of information about breast cancer and may challenge the current treatment protocols.

There are three major molecular types of breast cancer. Hormone receptor–positive (HR+) tumors express positivity for estrogen receptors (ERs), progesterone receptors (PRs), or both. HR+ cancers are further classified into the luminal A and luminal B subtypes, with luminal A cancers associated with a better prognosis than luminal B cancers.[3] Human epidermal growth factor receptor 2–positive (HER2+) breast cancer accounts for approximately 30% of all breast cancer cases.[1,2] Triple-negative breast cancer (TNBC) is, as the name suggests, negative for hormone receptors and is not associated with HER2 overexpression. Basal and claudin-low are the two molecular subtypes of TNBC; *BRCA1* and *BRCA2* mutations are associated with the basal subtype. The specific genetic mutations correlating to these molecular subtypes are highly variable.

Recent studies of the breast cancer genome have identified several molecular alterations in the phosphatidylinositol 3-kinase/protein kinase B/mechanistic target of rapamycin (PI3K/AKT/mTOR) pathway that are associated with the development of breast cancer.[1] PI3K/AKT pathway mutations have been identified in more than 30% of invasive breast cancers.[1] Genetic mutations can occur in several places to activate this pathway, from tyrosine kinase (TK) receptor mutation and amplification to gain-of-function mutations involving *PIK3CA* catalytic and regulatory subunits. The end result in each case is activation of the PI3K pathway, promoting tumor cell proliferation.[3]

The *PIK3CA* gene encodes the p110a catalytic subunit of the PI3K pathway.[1] Two regions of the *PIK3CA* gene

have been identified as mutational "hot spots"; mutations of *E542K* and *E545K* in the helical domain are associated with decreased survival and nodal involvement, while mutations of the H1047R allele in the catalytic subunit correspond to increased survival rates, comparatively.[1,3]

Other mutations of the PI3K pathway may occur with or without *PIK3CA* mutations; in fact, it is not uncommon for multiple mutations to be identified in a tumor.[3] These mutations include amplification and activation of tyrosine kinases (AKT1 and AKT2, in particular) and inactivation of PTEN.[3] Loss of PTEN expression has been noted in 30% of primary breast cancers.[3] The presence of several PI3K pathway mutations is associated with a poorer outcome.[3]

Thirty-five percent of ER+ tumors express *PIK3CA* mutations, and mutations of the PI3K pathway are associated with both acquired and de novo resistance to tamoxifen and aromatase inhibitors, the current treatment regimen for hormone receptor–positive tumors.[3,5] *PIK3CA* mutation is also associated with 23% of HER2+ tumors, and has been linked to resistance to trastuzumab and lapitinib.[3,5]

While *PIK3CA* mutations are most often associated with HR+ and HER2+ tumors, they have been identified in 8% of TNBC basal tumors, and they have also been linked to other types of breast cancer and precancerous lesions, including papillary carcinoma and ductal carcinoma in situ (DCIS).[3] In addition, *PIK3CA* mutations have been identified in 47% of metaplastic breast tumors.[3]

The identification of specific genetic mutations along the PI3K pathway has led to an investigation into the discovery of therapeutic agents that are developed to target these specific pathway mutations.[4] The addition of PI3K/AKT/mTOR inhibitors to aromatase inhibitor therapy has shown success in halting tumor growth. In the BOLERO-2 study, postmenopausal women with ER+ breast cancer that had progressed despite treatment with aromatase inhibitors were given either exemestane plus everolimus, an mTOR inhibitor, or exemestane plus placebo. Median progression free survival (PFS) was 10.6 months in the everolimus group versus 4.1 months in the exemestane-only group *(P* <.001).[5] A similar phase III trial, BOLERO-1, is currently under way, evaluating the efficacy of combination therapy with trastuzumab plus everolimus as a first-line treatment for HER2+ breast cancer.[5]

Currently, there are no approved first-line targeted therapeutic agents for TNBC. The complex pathology and genetic instability of this group of tumors has made it difficult to identify mechanisms of tumorigenesis, which, in turn, has impeded the development of targeted agents. This is especially true for the claudin-low tumor subtype, which is the most recent breast cancer subtype to be identified.[6] These tumors differ from other molecular subtypes, in that they express very low to nonexistent levels of luminal differentiation markers while expressing high levels of mesenchymal and stem cell markers.[6] Claudin-low tumors also show little to no expression of PI3K pathway activity.

Poly(ADP-ribose) polymerases (PARP) inhibitors have shown promise in the treatment of breast cancers with *BRCA1* and *BRCA2* mutations in phase II trials.[5] PARP inhibitors may also be effective in the treatment of ovarian tumors with *BRCA* mutations. The identification of *PIK3CA* mutations in basal tumors suggests that PI3K-targeted therapy may be beneficial in treating this type of TNBC. The pan-PI3K inhibitor BKM120 is being investigated in an ongoing phase II trial, and early trials of combination therapy with anti-PI3K pathway agents and PARP inhibitors are under way.[5]

Molecular Profiling of Colorectal Cancer

Colorectal cancer (CRC) represents the second leading cause of cancer death in the United States. Although more chemotherapeutic and biologic treatment options are available than in years past, and patient survival for metastatic CRC has improved more than twofold in the last 20 years, at least 136,000 new cases are anticipated along with more than 50,000 deaths due to CRC this year alone.[7]

Increasing evidence indicates that some mutations in CRC may serve as prognostic or predictive markers to guide decisions regarding specific therapies. Key genes that harbor such mutations include *KRAS, NRAS, BRAF, PIK3CA, PTEN, AKT1, SMAD4,* and *TGFbR2*.8 Importantly, many of these mutant gene products are potential targets for drug development.9 It is increasingly essential to understand how particular molecular features translate to differences in tumor biology, in order for targeted drug design to succeed.

RAS

In approximately 40% of CRC, constitutive activation of KRAS signaling pathways occurs via mutations in codons 12 and 13 of the *KRAS* gene, and *NRAS* mutations occur in approximately 3% of CRC.[9,10] Patients with *KRAS* or *NRAS* mutations do not benefit, and sometimes fare worse, from anti-EGFR therapy. For the traditional designation of wild-type *KRAS* (no mutations in codons 12/13), approximately 16% have clinically significant mutations in *KRAS* exon 3 and 4 and *NRAS* exons 1, 2, 3, and 4.[11] Based on these data, the National Comprehensive Cancer Network (NCCN) released updated guidelines to recommend testing metastatic CRC for mutations in both *KRAS* and *NRAS*; previous standard of care tested only exon 2 of *KRAS.*[12] Approximately 50% of all CRC has no mutations detected in exons 2, 3, or 4 of *KRAS* or *NRAS*.

It is imperative to further explore the molecular

pathology of CRC beyond *KRAS* in patient selection for effective anti-EGFR therapy. More-extensive genetic testing for *RAS* gene mutations beyond the routine analysis of *KRAS* exon 2 will become the standard of care for identifying patients appropriate to receive anti-EGFR therapy. This is supported by results from a comprehensive analysis of a large international cohort evaluating the prevalence of predictive molecular aberrations suspected of anti-EGFR therapy nonresponse in patients with wild-type *KRAS* revealing a mutation rate of 13.8% for *BRAF* and 40% for patients with either *PTEN* loss or a *PIK3CA* mutation.[13]

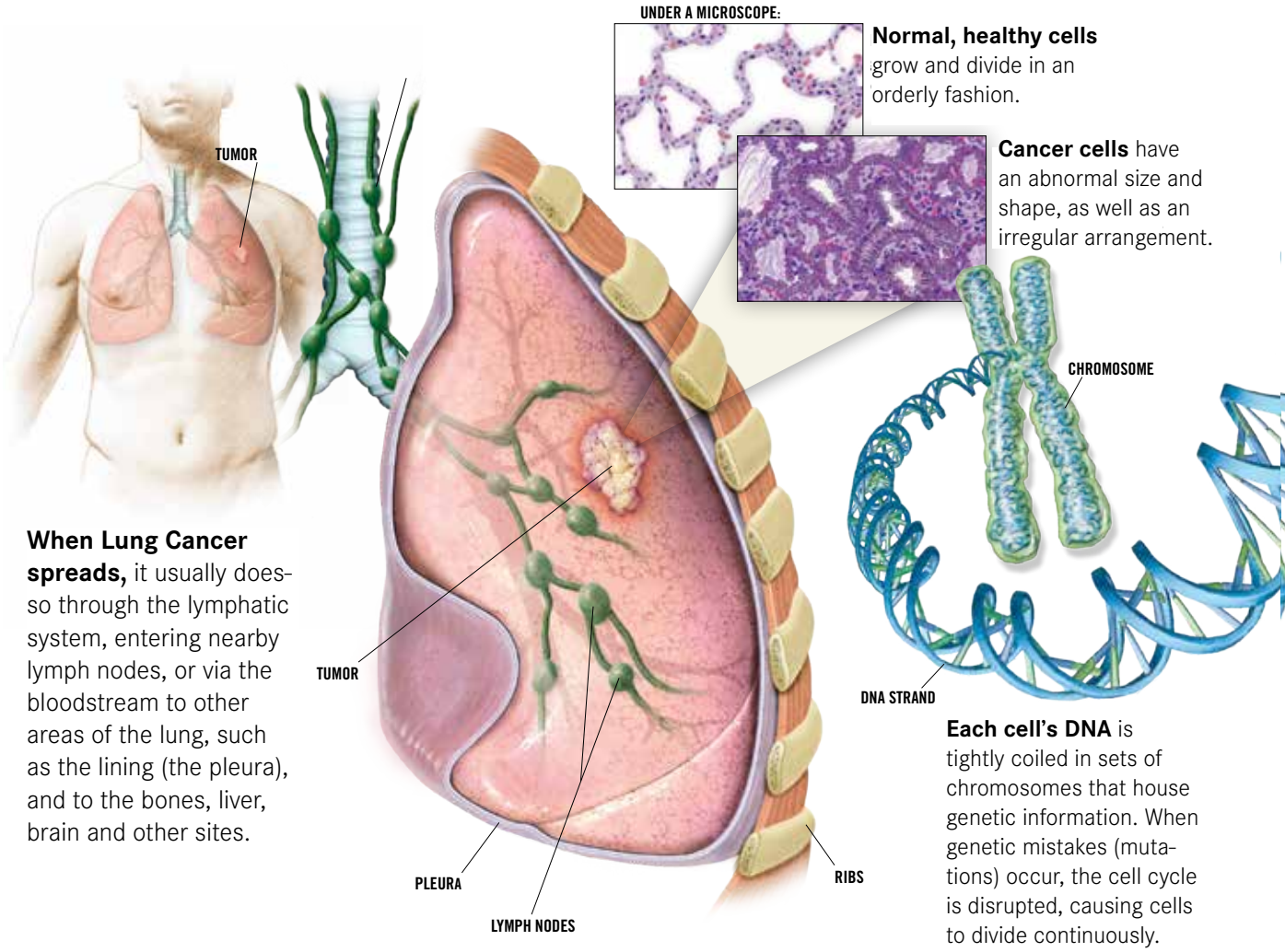
Expression of specific microRNAs also have the potential to serve as predictive biomarkers for therapeutic response, as evidenced by the expression of Hsa-miR-31-3p in patients with metastatic CRC with wild-type *KRAS* who are more likely to respond to anti-EGFR therapy.[14]

BRAF

RAF kinases are central mediators in the MAP kinase signaling cascade and act primarily through phosphorylation and activation of MEK. Approximately 5% of CRC tumors harbor *BRAF* mutations,[9] which exhibit a significant association with right-sided colon cancers and decreased overall survival.

PIK3CA

PIK3CA mutations exist in 15%-20% of CRC9,[15] and usually occur within two "hotspots" of exon 9 (helical domain) and exon 20 (kinase domain). New



When Lung Cancer spreads, it usually does so through the lymphatic system, entering nearby lymph nodes, or via the bloodstream to other areas of the lung, such as the lining (the pleura), and to the bones, liver, brain and other sites.

Each cell's DNA is tightly coiled in sets of chromosomes that house genetic information. When genetic mistakes (mutations) occur, the cell cycle is disrupted, causing cells to divide continuously.

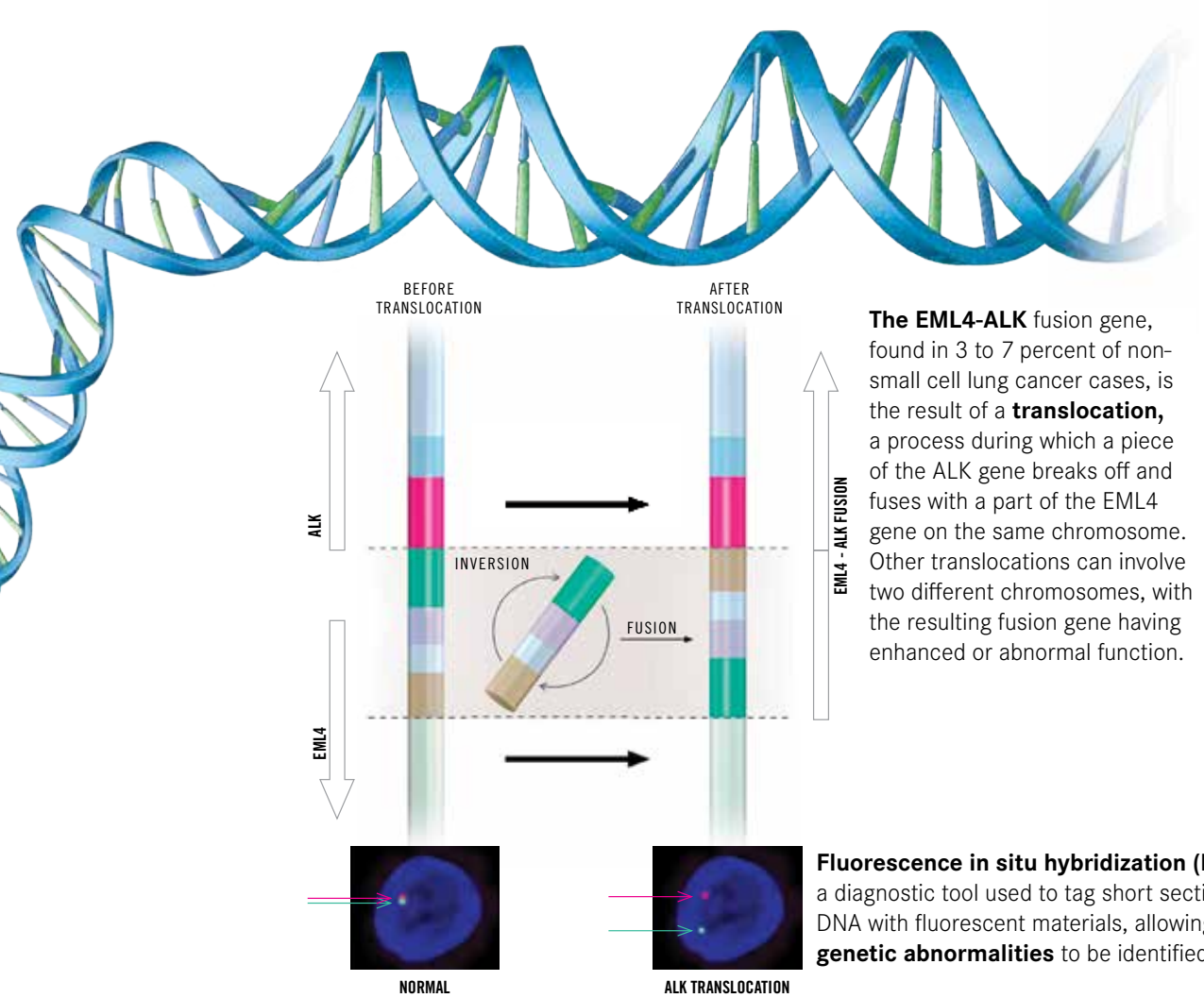
data suggest that *PIK3CA* mutations can serve as potential biomarkers for the benefit of aspirin in mid-stage CRC. Two studies of patients with mutations in *PIK3CA* who were regular aspirin users had a reduced CRC recurrence rate and a decreased probability of death, an effect not seen in patients with wild-type *PIK3CA* tumors.[15]

PTEN

The reported loss of PTEN expression in CRC widely varies from 19% to 36%[16]. PTEN loss, resulting in upregulation of the PI3K/AKT pathway, is associated with decreased sensitivity of CRC tumors to anti-EGFR treatment.[16] However, the role of *PTEN* mutation in CRC remains unclear, as some studies indicate an effect on response rate and survival, whereas others demonstrate implications only on progression-free or overall survival.17 Further research into the predictive significance of *PTEN* mutations and other mechanisms for loss of PTEN expression is ongoing; however, until standardized methods for PTEN expression analysis by immunohistochemistry are validated, PTEN expression cannot serve as a reliable tool for outcome analyses in CRC.

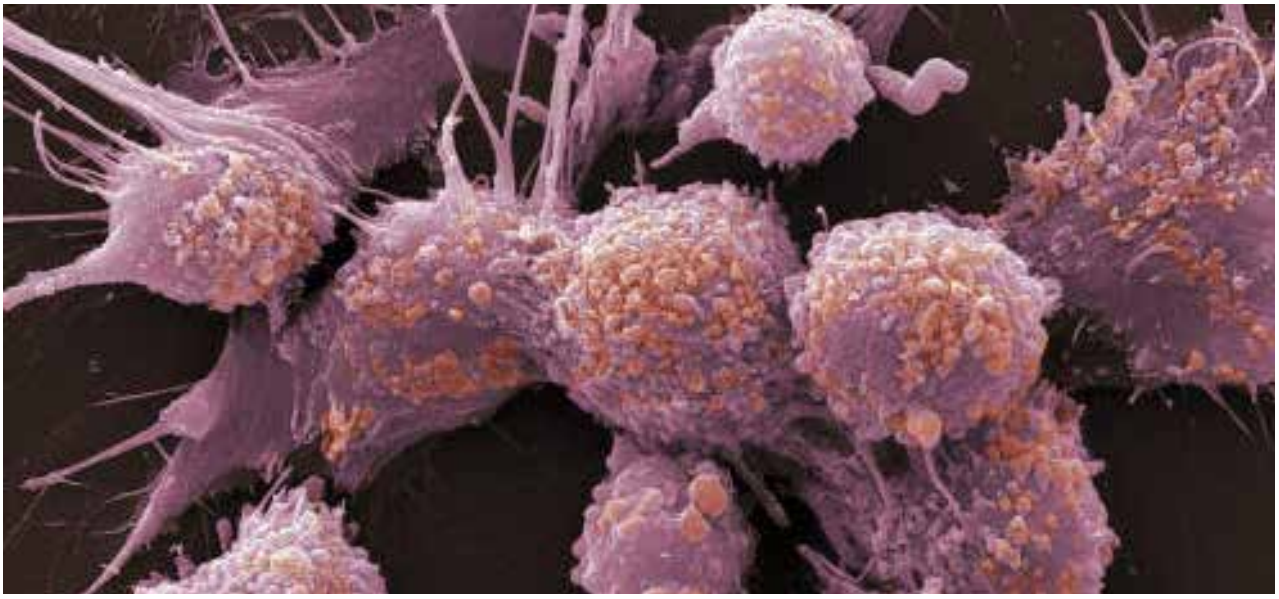
AKT1

Approximately 6% of all CRC has a mutation in *AKT1* (E17K), which occurs in the Pleckstrin homology



The EML4-ALK fusion gene, found in 3 to 7 percent of non-small cell lung cancer cases, is the result of a **translocation,** a process during which a piece of the ALK gene breaks off and fuses with a part of the EML4 gene on the same chromosome. Other translocations can involve two different chromosomes, with the resulting fusion gene having enhanced or abnormal function.

Fluorescence in situ hybridization (FISH) is a diagnostic tool used to tag short sections of DNA with fluorescent materials, allowing certain **genetic abnormalities** to be identified.



domain and leads to constitutive kinase activity due to alteration of the ligand binding site.[18] It remains unclear what specific clinical characteristics are associated with CRC patients harboring mutations in this gene. *AKT1* mutations and *PTEN* mutations appear to be mutually exclusive, as do *AKT1* and *PI3K* mutations.

TGFbR2 and SMAD4

Inactivating mutations in *TGFbR2* are associated with 30% of CRCs and contribute to malignant transformation of late adenomas. *SMAD4*, a post-TGFbR2 signaling pathway gene, is a tumor suppressor located on chromosome 18q in the region of most frequent (d70%) genetic alteration in CRC.[19] Loss of *SMAD4* immunostaining is observed in >50% of CRCs and is also associated with lymph node metastasis. Downregulation of SMAD4 is associated with worse survival in patients with early-stage CRC. Low *SMAD4* expression may also identify a subset of patients with early recurrence after curative therapy, and *SMAD4* expression levels may serve as a potential predictive biomarker for 5-fluorouracil (5-FU) therapeutic response.

TGFBR2 TGF

A recent two-tier genetic screen analysis of datasets containing normal mucosal tissues and CRC samples revealed the top eight differentially expressed genes, three of which were tumor suppressor genes not previously linked to CRC (*CA7*, *TCN1*, and *CWH43*).[20] Additionally, a genome-wide association study of CRC in East Asians recently identified six new loci associated with CRC risk, two of which map to genes with established roles in CRC (*TCF7L2* and *TGFb1*), but with four other loci located in or near genes involved in transcriptional regulation (*ZMIZ1*), genome maintenance (*FEN1*), fatty acid metabolism (*FADS1* and *FADS2*), cancer cell motility and metastasis (*CD9*), and cell growth and differentiation (*NXN*).[21]

Continuous discovery and further insight into the genetic basis of CRC hopefully will elucidate biological pathways that can potentially serve as critical drug targets.

Molecular Aberrations in Lung Cancer

Lung cancer is a prevalent disease in the United States and continues to affect thousands of Americans each year. The American Cancer Society estimates that 224,210 new cases of lung cancer will be diagnosed and 159,260 lung cancer-related deaths will occur in 2014, accounting for 27% of all cancer deaths.[22]

Lung cancer comprises both small-cell lung cancer (15%) and non-small-cell lung cancer (NSCLC) (85%), and nearly 70% of all patients with lung cancer present with locally advanced or metastatic disease at time of diagnosis.[23] While several targeted therapies have been developed for the treatment of NSCLC, the 5-year survival rate remains at 15%.[23] On average, patients with stage IIIB or IV lung cancer survive only 10 to 12 months.[23]

Although advanced NSCLC survival rates are low, targeted therapies have been developed for several different NSCLC subtypes defined by oncogenic driver mutation. The ability to identify molecular abnormalities in tumors of patients with NSCLC allows clinicians to better predict patient responses to several different types of therapies.[23] Treating individual tumors based on the molecular aberrations driving tumor growth should improve survival rates in patients with NSCLC.

Several molecular alterations have been detected in NSCLC adenocarcinomas. The most common are *KRAS* mutations, epidermal growth factor receptor (*EGFR*) mutations, and anaplastic lymphoma kinase (*ALK*) fusion proteins.[24] Targeted therapies have been developed for *EGFR* and *ALK* aberrations. However, no approved therapies are available specifically for *KRAS*-mutant lung cancer.[24]

KRAS mutation is the most common molecular aberration observed in NSCLC. It occurs in 15%-25% of all NSCLC adenocarcinomas and is usually present in current or former smokers who develop lung cancer.[24] *KRAS* mutations are typically missense mutations that result in constitutive activation of the KRAS signaling pathway, leading to uninhibited cell proliferation.[24] NSCLCs that contain *KRAS* mutations are usually wild-type for *EGFR* and *ALK*, indicating that *KRAS* mutations represent a distinct molecular subset of lung cancer.[24] Due to unsuccessful efforts to target *KRAS* mutations pharmacologically, very few clinical trials have been conducted solely for patients with *KRAS*-positive NSCLC.24 Thus, the prognostic and/or predictive value of *KRAS* for NSCLC is not known.[24] However, *KRAS* positivity in NSCLC is a negative predictor for response to erlotinib and gefitinib, two anti-EGFR tyrosine kinase inhibitors (TKIs).[24]

EGFR mutations are present in 10% of NSCLCs in the United States and are most commonly detected in female never-smokers who develop lung adenocarcinoma.[24] *EGFR* mutations result in amplified kinase activity and downstream signaling of the mitogen-activated protein kinase and phosphoinositide 3-kinase signaling pathways, which lead to cell proliferation and survival, respectively.[24] *EGFR* mutations and other common NSCLC molecular aberrations are not usually present simultaneously, identifying *EGFR* mutation as a distinct molecular subtype of NSCLC.[24] Several EGFR-targeted therapies have been developed to treat patients with mutated *EGFR*. These include the reversible EGFR TKIs gefitinib and erlotinib, the irreversible EGFR TKI afatinib, and monoclonal antibodies targeting EGFR.[24]

While gefitinib and erlotinib have shown dramatic clinical responses in patients with NSCLC with *EGFR* mutations, all *EGFR*-positive tumors develop resistance to these therapies.[25] Afatinib is a third-generation, irreversible EGFR TKI that has been shown to induce a robust clinical response in *EGFR*-positive tumors that are resistant to gefitinib and erlotinib.[26] For many patients (49%), resistance occurs as the result of an acquired mutation in *T790M*. To address this resistance mechanism, several third generation TKIs that bind selectively to *T790M* are being developed (AZD9291 and rociletinib), with response rates of approximately 60% in the second-line setting.

ALK fusion proteins occur in 3%-7% of lung tumors and are most commonly detected in younger patients who are light smokers or never-smokers.[24] Several different *ALK* fusion proteins have been recorded, each resulting in increased tyrosine kinase signaling followed by cell proliferation, survival, and decreased apoptosis.[24] Most *ALK* fusions are variants of echinoderm microtubule-associated protein-like 4 (EML4) fused with the *ALK* gene.[24] As with *EGFR* and *KRAS*, *ALK* fusions in lung cancer rarely occur with other molecular aberrations.[24] The first-generation ALK TKI crizotinib and second-generation ceritinib are two targeted therapies currently used for the treatment of patients with NSCLC with *ALK* fusions.[24] Ceritinib has been shown to be effective in patients whose tumors have developed resistance to crizotinib.[27]

While lung cancer remains prevalent in the United States, molecular profiling of lung tumors provides useful insight for treating individual patients with distinct drivers of tumorigenesis, including *KRAS*, *EGFR*, *ALK*, and several others. Targeted therapies to specific molecular aberrations have improved clinical response and progression-free survival in patients with known oncogenic drivers. Many tumors are negative for all known oncogenic drivers, so the identification of these drivers as well as the development of novel therapeutic agents will lead to improved survival in patients with lung cancer.

Genetic Profiling of Melanoma: Molecular Subgroups and Targeted Therapies

Melanoma, or cancer of melanocytes, is a common and deadly form of skin cancer affecting the US population, with 76,100 cases expected to be diagnosed, and over 9700 deaths projected in 2014. Patients with stage IV metastatic melanoma have a less than 10% chance of surviving beyond 5 years, and an urgent need exists for new and effective therapeutic strategies in these patients.[28]

The molecular analysis of melanomas for the presence of "driver" oncogenic mutations has proved to be important both from a prognostic and a therapeutic point of view. Melanomas are now being classified into molecular subgroups, and genetic profiling has enabled the development of crucial targeted therapies for melanoma.

The main driver mutations activate signaling pathways or may directly affect cell cycle progression. Mutations in *BRAF*, *NRAS*, *GNA11*, *GNAQ*, *KIT*, and *MEK1* activate the MAP kinase signaling pathway and are found in nearly 70% of melanomas.[28] *CTNNB1* mutations activating the

Wnt signaling pathway, and *CDKN2A* and *CDK4* mutations altering cell cycle are somewhat less common; however, in tumors lacking *NRAS* or *BRAF* mutations, two or more cell cycle–related mutations may coexist, indicating that additional events are required to deregulate the cell cycle.[28,29]

Melanomas that are classified on their histologic and anatomic bases differ in their genetic profiles. Cutaneous melanomas arising from chronic sun damage have a much lower frequency of *BRAF* mutations (10% *BRAF*, 10% *NRAS*, 2% *KIT*) than those without chronic sun damage (50% *BRAF*, 20% *NRAS*). Mucosal melanomas (5% *BRAF*, 15% *NRAS*, 20% *KIT*), and acral melanomas (15% *BRAF*, 15% *NRAS*, 15% *KIT)* have a higher incidence of *KIT* mutations. Uveal melanomas have a distinct genetic profile (32% *GNA11*, 50% *GNAQ*, <1% *BRAF*), distinguishing them from the other subtypes.[28] The various gene mutations and the evolving targeted therapies are discussed below.

BRAF functions downstream of *RAS* and leads to activation of ERK1. By far the most prominent and relevant of all driver mutations, *BRAF* mutations are found in approximately 50% of all malignant melanoma cases and in approximately 59% of melanomas not induced by chronic sun damage.[30] The most frequently reported mutations are *V600E* (80%-90% cases), *V600K* (5%-12% cases), and *V600R* or *V600D* (<5%).[29,30] The presence of *BRAF* mutations are associated with poor prognosis, and *BRAF* inhibitors, targeted against *V600* mutations (vemurafenib, dabrafenib), are among the most effective targeted therapies currently approved for *BRAF*-mutated melanoma.

Next to *BRAF*, mutations in *NRAS*, a *RAS* family oncogene, are most common, found in 15%-20% of all malignant melanomas. As with *BRAF*, mutations in *NRAS* are associated with poor prognosis in metastatic melanomas, but unlike *BRAF*, these are found more commonly in chronic sun damaged melanomas.[28] The most common mutations are Q61, G12, and G13, all of which lead to GTPase inhibition, which constitutively activates downstream MAP kinase pathways. *NRAS*-mutated tumors are currently targeted by MEK inhibitors, which function downstream in the RAS/RAF/MEK/ERK pathway.[29]

Mutations or amplifications of KIT, a type III transmembrane receptor tyrosine kinase, is more common in mucosal and acral melanomas (10%-20%) and can activate multiple downstream signaling pathways such as MAP kinase, PI3K/AKT, and JAK/STAT. In recent clinical trials, melanomas with *KIT* alterations are responsive to treatment with imatinib. Other KIT inhibitors such as sunitinib, dasatinib, and nilotinib have shown limited success.[29]

GNAQ and *GNA11* are members of the alpha G protein family, and mutations in these genes occur in 80%-90% of uveal melanomas. All mutations in these proteins lead to inhibition of GTPase activity, leading to constitutive activation.[29]

MEK1 mutations are found in approximately 6% of all malignant melanomas; however, the distribution in various subtypes in unknown.[31] In many tumors, *MEK1* mutations coexist with *BRAF* mutations, and these mutations are thought to contribute to *BRAF* inhibitor resistance. Phase III trials of *MEK1* inhibitors have shown promising results in treating *BRAF*-mutant melanomas when used in combination with *BRAF* inhibitors. In addition, MEK inhibitors are among the first targeted therapies that have shown benefit in *NRAS*-mutant melanomas.[29]

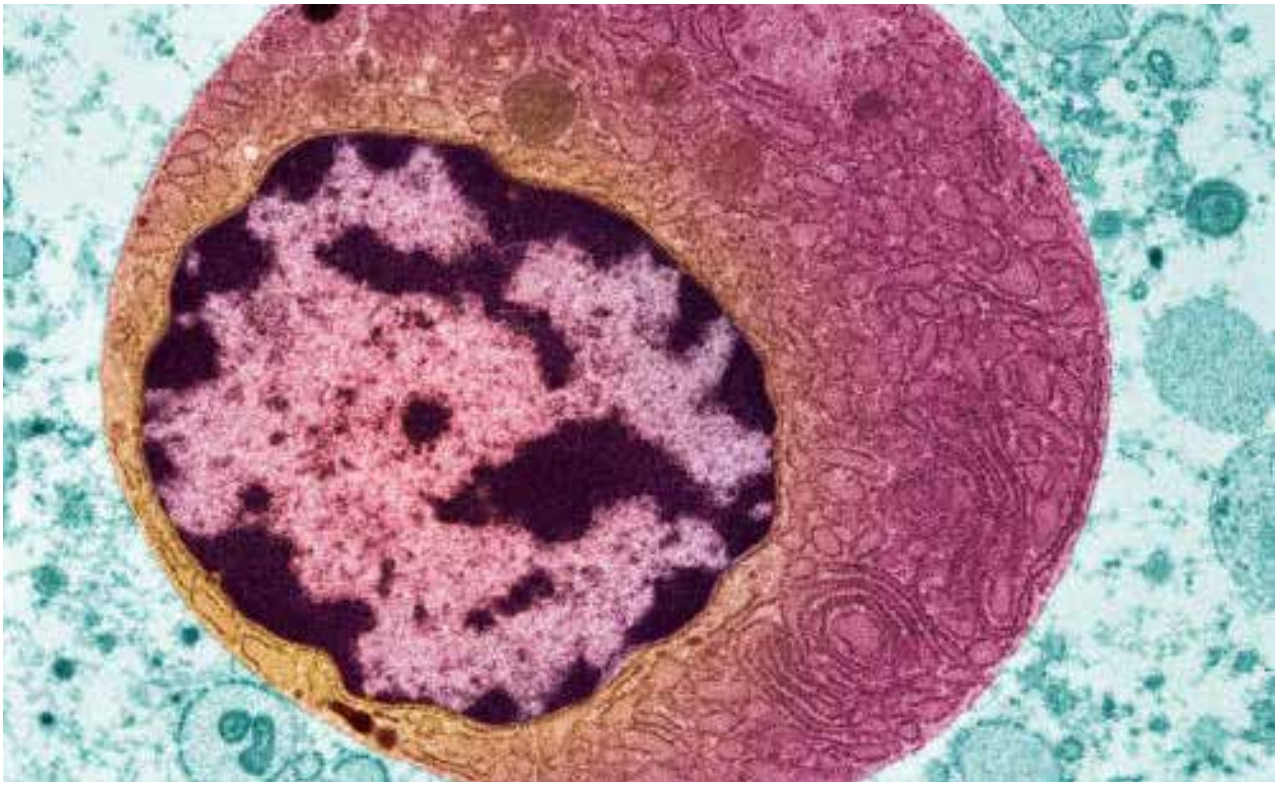
Activating mutations in *CTNNB1* are reported in 2%-3% of melanomas, although varying percentages have been reported in other studies, and *CTNNB1* mutations are rare in uveal melanomas. *CDKN2A* mutations are frequently genetic rather than somatic events, and are reported in 8% to 57% of familial melanoma cases.[29,32,]

According to a recent report, multiple mutations occur in 13% of all cutaneous melanomas, the most co-occurring mutations being *BRAF*, *NRAS*, and *CDKN2A*.33 These findings are important and relevant to understanding resistance to current targeted therapies. The most common reasons for resistance to BRAF inhibitors are additional mutations in the MAP kinase pathways (*NRAS* and *MEK*), upregulation of cMET tyrosine kinase receptor, and *CCND1* overexpression.[29] Resistance to KIT inhibitor therapy is associated with mutations in *NRAS* and activation of the PI3K/AKT/mTOR pathway.[29] Combination therapy is the latest emerging trend in melanoma therapeutics, and it has shown promise in treatment of metastatic melanomas. Molecular profiling will continue to hold the key to the development of future effective therapies to combat this deadly skin cancer.

Genetic Profiling Enhances Understanding of Prostate Cancer

Prostate cancer is the second leading cause of cancer death in men.[34] Estimates for prostate cancer in the United States for 2015 are about 220,800 new cases and about 27,540 deaths. One in seven men will be diagnosed in his lifetime, and prostate cancer appears to run in some families[34,35]: A person has twice the risk of developing prostate cancer if he has a brother or father with prostate cancer.[35]

Genetic profiling is used to gain information about the genes involved in prostate cancer. The goal is to learn about genetic changes that lead to prostate cancer, and develop tests and treatments to detect and fight this cancer.[36] There are no tests for the specific



genes that indicate an increased risk for inherited prostate cancer; however, several known biomarkers are used to characterize prostate tumors and provide diagnostic and prognostic information.[36,37]

TMPRSS2-ERG is a combination of two genes that together cause cancer cells to be activated by hormones in the prostate.[37] *TMPRSS2-ERG* signifies highly aggressive cancer and is discovered in 50% of prostate cancers.[37]

PTEN is a gene responsible for producing a protein that blocks a pathway that restrains cell growth and survival.[37] Some prostate cancer cells lose one or both copies of this gene, causing cells to proliferate faster than normal.[37] The *PTEN* gene occurs in approximately 40% of prostate cancers.[37]

A protein, prostate-specific antigen (PSA), is produced by normal and cancerous prostate cells.[37] PSA is found in blood, generally at low levels in healthy men and higher levels in men with a higher risk of prostate cancer.[37] The chance of having prostate cancer increases with a higher PSA level.[4] Monitoring PSA levels enables doctors to assess the risk of prostate cancer development, determine recurrence, and decide whether the cancer has stopped responding to treatment.[37]

Prostate cancer gene 3 (*PCA3*) is a diagnostic biomarker for prostate cancer.[37] *PCA3* produces an RNA molecule found in high levels in prostate cancer.[37] *PCA3* is found in significant amounts in 95% of patients with prostate cancer.[37]

A protein, Ki-67, is expressed in large amounts in prostate cancer cells that are growing and dividing.[37] Ki-67 is a prognostic biomarker for prostate cancer, given that a large production of Ki-67 indicates an increased chance of metastases and recurrence, as well as a worse prognosis.[37]

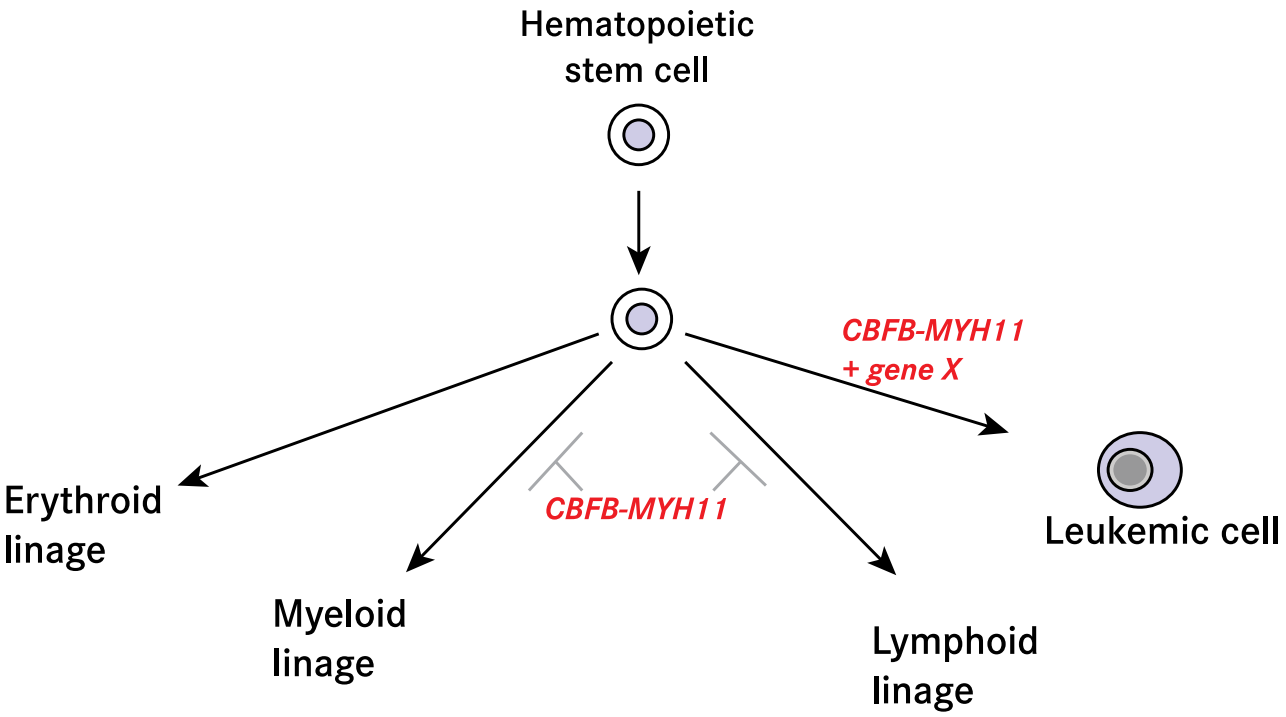
MicroRNAs have also recently been shown to be an important factor in prostate cancer.[38] MicroRNAs are short nucleotide sequences that may help with early diagnosis of prostate cancers with poor prognosis.[38]

Continued discoveries in genetic profiling should enable enhanced screening, diagnostics, and treatments and therapies for prostate cancer.

Genetic Profiling Expands Treatment Options for Ovarian Cancer

Ovarian cancer is the deadliest type of gynecological cancer in the United States, with 14,270 deaths estimated in 2014.[43] Many factors, including late-stage diagnosis and relapse after surgery and chemotherapy, contribute to its high mortality.[44]

While once regarded and treated as a single disease, ovarian cancer is now classified as a heterogenous disease. Ovarian tumors are classified into two types or grades based on their morphological and clinical characteristics.[45-47] Type I or low-grade are serous, clear-cell, endometrioid, or mucinous tumors that are slow growing, noninvasive, and relatively resistant



Expression of CBFB-MYHii fusion gene in the hematopoietic stem cellls blocks differentiation in the myeloid and lymphoid lineages. Additional gene alterations can bypass the block and collaborate with CBFB-MY11 to develop leukemia

to chemotherapy. By contrast, type II or high-grade serous tumors are rapidly growing, highly invasive, and represent advanced-stage disease. Seventy-five percent of women diagnosed with ovarian cancer have type II tumors.[48]

Genetic profiling of type I and type II ovarian tumors has revealed that each tumor type has its own molecular signature.[45,47,48-50] Type I ovarian tumors harbor mutations in a number of different genes including *BRAF, KRAS*, *PTEN,* and *beta-catenin*. whereas type II ovarian tumors do not. Mutations in *TP53* and *BRCA1/2*, which are fairly common in type II ovarian tumors, are rarely found in type I. Although both tumor types display enhanced PI3K/Akt signaling, in type I tumors it is the result of somatic mutations in *PTEN* and *PI3KCA,* and in type II tumors it is the result of increased copy numbers of *PI3KCA* and *AKT1/2/3*.

The distinct molecular signatures of type I and type II ovarian tumors suggest different therapeutic targets for each type of tumor, many of which are now being investigated in clinical trials.[49-53] Inhibiting the KRAS signaling pathway, the PI3K signaling pathway, or both, may be effective in treating type I ovarian tumors. For type II tumors, inhibition of the BRCA-mediated DNA repair pathway, either alone or in combination with inhibition of the PI3K pathway, may hold promise.

In December 2014, the FDA approved the PARP inhibitor olaparib (Lynparza) as a treatment for women with BRCA-positive advanced ovarian cancer following three or more prior lines of chemotherapy. Outside of this, the most common "actionable" alterations with potential for small-molecule targeted therapy in epithelial ovarian cancer are in the PIK3CA/PTEN and KRAS/BRAF signaling pathways.

Genetic profiling of type I and type II ovarian tumors has expanded the options in the treatment of ovarian cancer. By tailoring the treatment to the type of ovarian tumor, the mortality rates for ovarian cancer are expected to significantly improve.

Genetic Profiling in Leukemia

Major types of leukemia distinguished by cell type and growth characteristics include acute myelogenous leukemia (AML), chronic myeloid leukemia (CML), and acute lymphocytic leukemia (ALL).

AML, a heterogeneous group of myeloid neoplasms, is the most common type of acute leukemia in adults, with an estimated 8860 new diagnoses and 10,460 deaths in the United States in 2014.[39] AML derives from the clonal expansion of hematopoietic progenitors of the bone marrow. Pathogenesis is driven by sequential acquisition of an initiating growth promoting mutation followed by additional cooperating mutations, resulting

in neoplastic transformation.[40] Among many recurrent somatic mutations identified in AML, *FLT3*, *NPM1*, and *CEBPA* mutations have proven prognostic value and guide risk stratification and treatment approaches combined with cytogenetic markers.[40] Targeted agents in development for AML that are based on recurrent mutations include inhibitors of the FLT3, JAK2, MEK, KIT, PLK1 kinases, and DOT1L inhibitors for AML with *MLL* rearrangements.[40] Hypomethylating agents used for AML patients not eligible for chemotherapy may be more effective in AML with mutations in epigenetic modifiers, including *IDH1*, *IDH2*, and *DNMT3A*.[40]

Chronic myeloid leukemia (CML) accounts for approximately 15% of adult leukemia; for 2014, estimates project 5980 new cases and 810 deaths.[39] CML is defined by the presence of a reciprocal translocation between chromosomes 9 and 22, encoding the chimeric BCR-ABL1 tyrosine kinase. Constitutive activity of BCR-ABL1 in hematopoietic stem cells leads to oncogenic transformation through increased cellular proliferation, resistance to apoptosis, and genetic instability.[41] Treatment with targeted tyrosine kinase inhibitors (TKIs) that block BCR-ABL1 activity has increased 10-year survival rates to 90%.[41] Frequent monitoring of response to treatment by quantitative assessment of BCR-ABL transcript is essential to identify resistance to TKI treatment, which is most frequently caused by recurrent point mutations in the BCR-ABL1 kinase domain. Mutational analysis aids in the choice of subsequent therapy for patients with inadequate initial response to first-line or second-line TKI therapy.[41]

ALL is the most common malignancy in children, accounting for 75% to 80% of pediatric leukemia and for 26% of all childhood cancers.[39] Approximately 6020 new diagnoses of ALL are expected in the United States in 2014.39 ALL is characterized by aberrant proliferation and survival of immature hematopoietic progenitors; their immunophenotypic and histologic properties define precursor B-cell, mature B-cell, T-cell ALL, and subtypes thereof.[42]

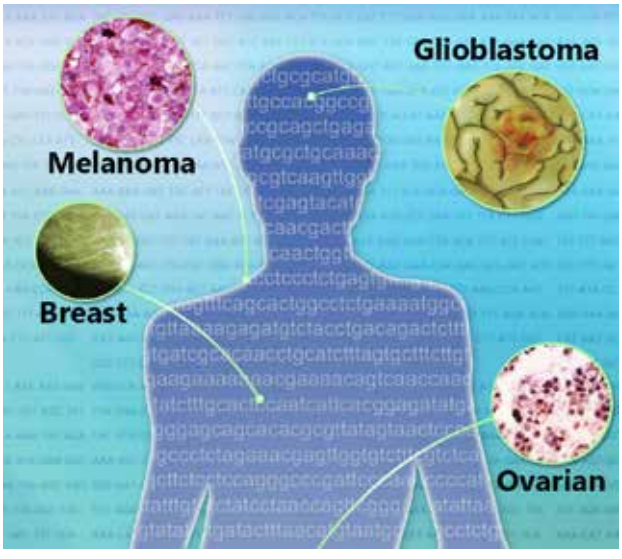
Evaluation of a small number of recurrent molecular abnormalities in specific ALL subtypes are in clinical use for risk stratification, including the favorable *TEL-AML1* (*ETV6-RUNX1*) fusion gene in pediatric B-cell ALL, and poor risk markers such as *MLL* rearrangements and *BCR-ABL1*. In the latter, and in patients with Philadelphia-like ALL, characterized by mutations in the Ras and JAK/STAT pathways, TKI therapy may improve outcomes.[42]

Mapping the Thyroid Cancer Genome

Cancers of the thyroid are the most commonly diagnosed malignancies of endocrine etiology, and they are the number one cause of death among endocrine cancers. It is estimated that in 2014, 62,980 new cases of thyroid cancer will be diagnosed, and there will be 1890 deaths attributed to the disease.[54] Thyroid cancer is three times more common in women than men, and two-thirds of all cases are diagnosed in people under the age of 55 years.[55] Thyroid cancer is the fastest growing cancer diagnosis in the United States; this increase can in large part be attributed to earlier and more accurate detection of thyroid tumors using ultrasound-guided biopsy and fine-needle aspiration.[55]

Two types of thyroid parenchymal cells give rise to thyroid cancer. Follicular cells form the lining of colloid follicles; these cells produce and secrete thyroid hormones. Well-differentiated thyroid cancer originates from follicular cells; this includes papillary (PTC), follicular (FTC), and Hürthle cell cancer. Follicular cells can also give rise to poorly differentiated (PDTC) and anaplastic thyroid cancer (ATC).[55] Parafollicular cells are the origin of medullary thyroid cancer (MTC), which may occur either sporadically or familially.[55]

PTC is the most commonly diagnosed thyroid cancer, comprising up to 85% of all cases.[54] An acquired mutation of the *RET* gene, called the *PTC* oncogene, has been linked to 10%-30% of PTC cases; the presence of the oncogene is higher in children and in PTC that is associated with radiation exposure.[55] Mutation of the *BRAF* gene is common



As The Cancer Genome Atlas (TCGA) research papers were published on individual cancers - glioblastoma, breast, ovarian and others - and genomic data started to accumulate, investigators began noticing similarities across cancers, patterns in the chaos. The Pan-Cancer initiative, launched in 2012 as a next logical step in TCGA studies, allows scientists to find a new way to pool all of this information and see commonalities among disease types.

in papillary tumors that are negative for the *PTC* oncogene; this genetic mutation is associated with higher rates of metastasis.[56]

FTC is responsible for 10%-15% of thyroid cancer diagnoses[54]; approximately 50% of these tumors are associated with activation of the *RAS* oncogene. This mutation is also noted in about 50% of cases of follicular adenoma, although currently there is no definitive evidence that *RAS*-positive adenomas will ultimately progress to FTC.[56]

BRAF and *RAS* mutations have also been identified in PDTC, which may indicate that some of these tumors arise from differentiated tumors. In addition, mutation of *p53* resulting in *TP53* deregulation has been noted in cases of PDTC and ATC.[56]

RET mutations associated with MTC occur at a different location on the gene than PTC *RET* mutations. About 10% of sporadic medullary tumors have a *RET* mutation. Familial MTC, which is associated with the syndromes MEN2a and MEN2b, is intrinsically linked to *RET* mutation. In sporadic MTC, the mutation is expressed only in the tumor cells; in familial tumors, one of the two *RET* genes is mutated in all cells, conferring a 50% change of inheritance.[56]

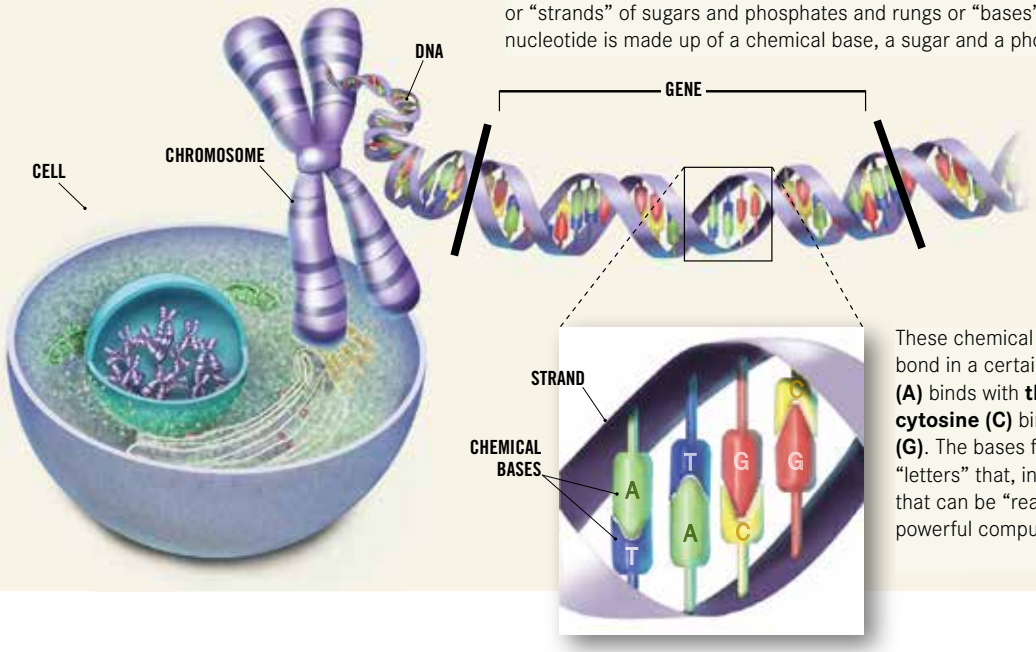
REFERENCES

1. Molecular profiling of breast cancer. *My Cancer Genome.* http://www.mycancergenome.org/content/disease/breast-cancer/. Accessed November 12, 2014.
2. American Cancer Society. Cancer facts and figures 2014. http://www.cancer.org/acs/groups/content/@research/documents/webcontent/acspc-042151.pdf. Accessed November 13, 2014.
3. Adams JR, Schachter NF, Liu JC, et al. Elevated PI3K signaling drives multiple breast cancer subtypes. *Oncotarget*. 2011;2(6):435-447.
4. Ellis MJ. Mutational analysis of breast cancer: guiding personalized treatments. *Breast.* 2013;22(suppl 2):S19-S21.
5. Mohamed A, Krajewski M, Cakar B, Ma CX. Targeted therapy for breast cancer. *Am J Pathol.* 2013;183(4):1096-1112.

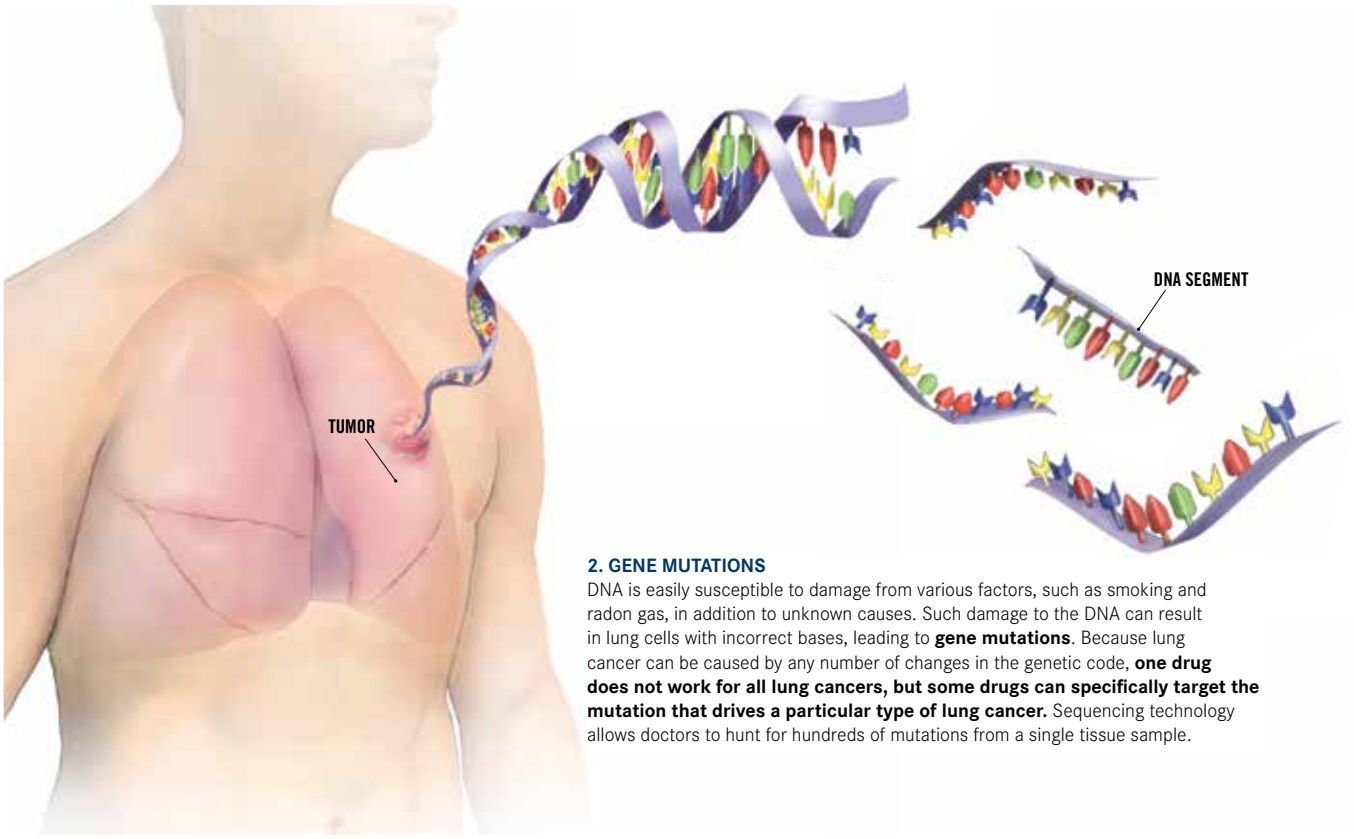
Genetics of Cancer

1. GENETICS 101

Nearly every cell in the body contains DNA (deoxyribonucleic acid). This genetic code is formed by a double helix, a twisted ladder-like structure with sides or "strands" of sugars and phosphates and rungs or "bases" of chemicals. A nucleotide is made up of a chemical base, a sugar and a phosphate.

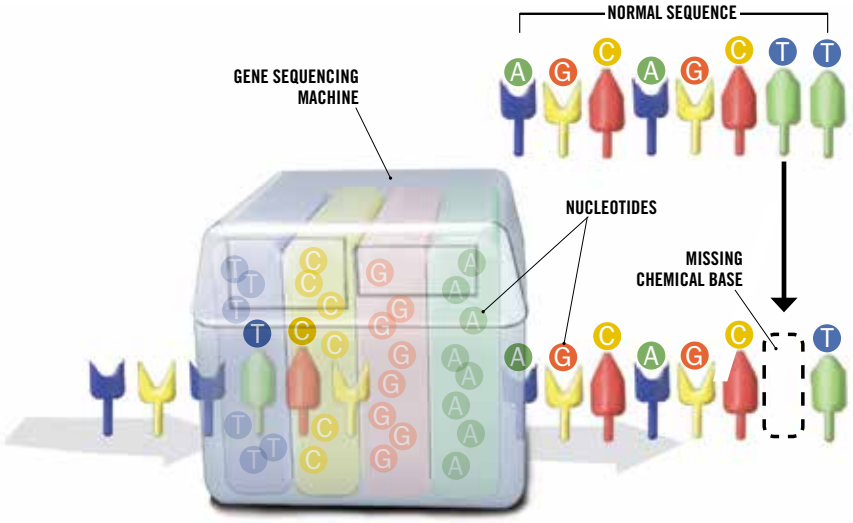


These chemical bases can only bond in a certain way: a**denine (A)** binds with **thymine (T)** and **cytosine (C)** binds with **guanine (G)**. The bases form strings of "letters" that, in turn, form "words" that can be "read" as genes by powerful computers.



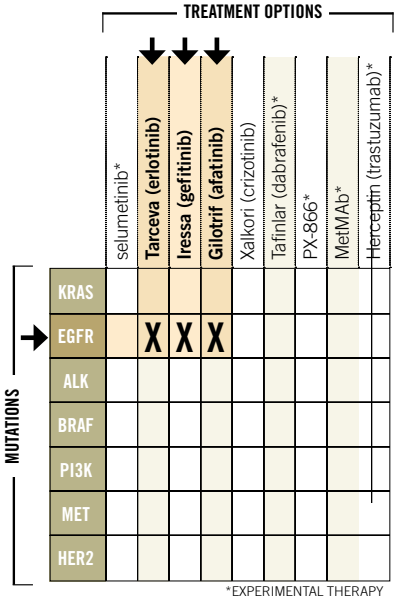
2. GENE MUTATIONS

DNA is easily susceptible to damage from various factors, such as smoking and radon gas, in addition to unknown causes. Such damage to the DNA can result in lung cells with incorrect bases, leading to **gene mutations**. Because lung cancer can be caused by any number of changes in the genetic code, **one drug does not work for all lung cancers, but some drugs can specifically target the mutation that drives a particular type of lung cancer.** Sequencing technology allows doctors to hunt for hundreds of mutations from a single tissue sample.



3. GENE SEQUENCING

Essentially, the sequencing process starts when a DNA sample is placed in a machine that **bathes it with one of the four DNA nucleotides**. As the DNA molecules make contact with their complementary nucleotide, the DNA pieces can be **deciphered and assembled into a readable code**, revealing missing or damaged elements.



4. TREATMENT

Using this information, doctors can sometimes **target the right drug to a specific mutation**. Although many lung cancer mutations have been discovered during the past decade, one of the first, **EGFR (epidermal growth actor receptor)**, revealed the possibilities for precision medicine using EGFR inhibitors.

6. Prat A, Parker JS, Karginova O, et al. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. *Breast Cancer Res.* 2010;12(5):R68.
7. American Cancer Society. Cancer facts and figures 2014. http://www.cancer.org/research/cancerfactsstatistics/cancerfactsfigures2014/. Accessed January 21, 2015.
8. Chan E. Molecular profiling of colorectal cancer. *My Cancer Genome.* http://www.mycancergenome.org/content/disease/colorectal-cancer/ (updated March 31); 2014. Accessed January 21, 2015.
9. De Roock W, Claes B, Bernasconi D, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol.* 2010;11(8):753-762.
10. Douillard JY, Oliner KS, Siena S, et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med.* 2013;369:1023-1034.
11. Peeters M, Oliner KS, Price TJ, et al. Analysis of KRAS/NRAS mutations in phase 3 study 20050181 of panitumumab (pmab) plus FOLFIRI versus FOLFIRI for second-line treatment (tx) of metastatic colorectal cancer (mCRC).Presented at: the 2014 Gastrointestinal Cancers Symposium; January 16-18, 2014; San Francisco, CA. Abstract LBA387.
12. National Comprehensive Cancer Network. Clinical Practice Guidelines in Oncology: Colorectal Cancer. http://www.nccn.org/professionals/physician_gls/recently_updated.asp. 2014. Accessed January 21, 2015.
13. Basu GD, Xiu J, Arguello D, et al. Prevalence of KRAS, BRAF, NRAS, PIK3CA, and PTEN alterations in colorectal cancer: analysis of a large international cohort of 5,900 patients. *J Clin Oncol.* 2014;32(suppl 3; abstr 399).
14. Manceau G, Imbeaud S, Thiebaut R, et al. Hsa-miR-31-3p expression is linked to progression-free survival in patients with KRAS wild-type metastatic colorectal cancer treated with anti-EGFR therapy. *Clin Cancer Res.* 2014;20:3338-3347.
15. Liao X, Lochhead P, Nishihara R, et al. Aspirin use, tumor *PIK3CA* mutation, and colorectal-cancer survival. *N Engl J Med.* 2012;367:1596-1606.
16. Salmena L, Carracedo A, Pandolfi PP. Tenets of PTEN tumor suppression. *Cell.* 2008;133(3):403-414.
17. De Roock W, De Vriendt V, Normanno N, et al. KRAS, BRAF, PIK3CA, and PTEN mutations: implications for targeted therapies in metastatic colorectal cancer. *Lancet Oncol.* 2011;12(6):594-603.
18. Carpten JD, Faber AL, Horn C, et al. A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. *Nature.* 2007;448(7152):439-444.
19. Grady WM, Pritchard CC. Molecular alterations and biomarkers in colorectal cancer. *Toxicol Pathol.* 2014;42:124-139.
20. Chu C-M, Yao C-T, Change Y-T, et al. Gene expression profiling of colorectal tumors and normal mucosa by microarrays meta-analysis using prediction analysis of microarray, artificial neural network, classification, and regression trees. *Dis Markers.* 2014;2014:634123.
21. Zhang B, Jia W-H, Matsuda K, et al. Large-scale genetic study in East Asians identifies six new loci associated with colorectal cancer risk. *Nat Genet.* 2014;46:533-542.
22. American Cancer Society. Cancer facts and figures 2014. 2014. http://www.cancer.org/acs/groups/content/@research/documents/webcontent/acspc-042151.pdf. Accessed November 16, 2014.
23. Molina JR, Yang P, Cassivi SD, et al. Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. *Mayo Clin Proc.* 2008;83(5):584-594. doi:10.4065/83.5.584.
24. Vanderbilt Ingram Cancer Center. *My Cancer Genome.* Genetically Informed Cancer Medicine. http://www.mycancergenome.org/content/disease/lung-cancer/. Accessed November 16, 2014.
25. Tartarone A, Lazzari C, Lerose R, et al. Mechanisms of resistance to EGFR tyrosine kinase inhibitors gefitinib/erlotinib and to ALK inhibitor crizotinib. *Lung Cancer (Amsterdam, Netherlands).* 2013;81(3):328-336. doi:10.1016/j.lungcan.2013.05.020.
26. Janjigian YY, Smit EF, Groen HJM, et al. Dual inhibition of EGFR with afatinib and cetuximab in kinase inhibitor-resistant EGFR-mutant lung cancer with and without T790M mutations. *Cancer Discov.* 2014;4(9):1036-1045. doi:10.1158/2159-8290.CD-14-0326.
27. Friboulet L, Li N, Katayama R, et al. The ALK inhibitor ceritinib overcomes crizotinib resistance in non-small cell lung cancer. *Cancer Discov.* 2014;4(6):662-673. doi:10.1158/2159-8290.CD-13-0846.
28. Vanderbilt Ingram Cancer Center. Molecular Profiling of Melanoma. *My Cancer Genome.* http://www.mycancergenome.org/content/disease/melanoma/ Updated August 8, 2014. Accessed November 16, 2014.
29. Griewank KG, Scolyer RA, Thompson JF, et al. Genetic alterations and personalized medicine in melanoma: progress and future prospects. *J Natl Cancer Inst*. 2014;106(2):djt435.
30. Vanderbilt Ingram Cancer Center. BRAF Mutations in Melanoma. *My Cancer Genome.* http://www.mycancergenome.org/content/disease/melanoma/BRAF/. Updated August 7, 2014. Accessed November 16, 2014.
31. Nikolaev SI, Rimoldi D, Iseli C, et al. Exome sequencing identifies recurrent somatic MAP2K1 and MAP2K2 mutations in melanoma. *Nat Genet*. 2011;44(2):133-139.
32. Vanderbilt Ingram Cancer Center. CTNNB1 (beta-catenin) Mutations in Melanoma. *My Cancer*

Genome. http://www.mycancergenome.org/content/disease/melanoma/CTNNB1 Updated October 24, 2012. Accessed November 16, 2014.
33. Yaman B, Akalin T, Kandiloğlu G. Clinicopathological characteristics and mutation profiling in primary cutaneous melanoma [published online ahead of print October 29, 2014]. *Am J Dermatopathol*. 2014. doi: 10.1038/ng.1026.
34. American Cancer Society. What are the key statistics about prostate cancer? http://www.cancer.org/Cancer/ProstateCancer/DetailedGuide/prostate-cancer-key-statistics. Accessed November 12, 2014.
35. Memorial Sloan Kettering Cancer Center. Inherited risk for prostate cancer. http://www.mskcc.org/cancer-care/hereditary-genetics/inherited-risk-prostate. Accessed November 12, 2014.
36 American Cancer Society. What's new in prostate cancer research and treatment? http://www.cancer.org/cancer/prostatecancer/detailedguide/prostate-cancer-new-research. Accessed November 12, 2014.
37. CancerCommons. Top biomarkers. http://www.cancercommons.org/patients-caregivers/prostate-cancer/top-biomarkers. Accessed November 12, 2014.
38. Wen X, Deng F-M, Wang J. MicroRNAs as predictive biomarkers and therapeutic targets in prostate cancer. *Am J Clin Exp Urol.* 2014;2(3):219-230.
39. American Cancer Society: Cancer facts and figures 2014. http://www.cancer.org/research/cancerfactsstatistics/cancerfactsfigures2014/. Accessed January 22, 2015.
40. Hatzimichael E, Georgiou G, Benetatos L, Briasoulis E. Gene mutations andmolecularly targeted therapies in acute myeloid leukemia. *Am J Blood Res.*2013;3(1):29-51.
41. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®): Chronic Myelogenous Leukemia. 2014. http://www.nccn.org/professionals/physician_gls/pdf/cml.pdf. Accessed November 13, 2014.
42. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®): Acute Lymphoblastic Leukemia. 2014. http://www.nccn.org/professionals/physician_gls/pdf/all.pdf. Accessed November 13, 2014.
43. National Cancer Institute: Surveillance, Epidemiology, and End Results (SEER) Program. http://seer.cancer.gov/statfacts/html/ovary.html. Accessed November 14, 2014.
44. Bast RC Jr, Hennessy B, Mills GB. The biology of ovarian cancer: new opportunities for translation. *Nat Rev Cancer*. 2009;9:415-428.
45. Shih I-M, Kurman RJ. Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. *Am J Pathol*. 2004;164:1511-1518.
46. Malpica A, Deavers MT, Lu K, et al. Grading ovarian serous carcinoma using a two-tier system. *Am J Surg Pathol.* 2004;28:496-504.
47. Kurman RJ, Shih I-M. Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer – shifting the paradigm. *Hum Pathol*. 2011;42:918-931.
48. Kurman RJ, Visvanathan K, Roden R, et al. Early detection and treatment of ovarian cancer: shifting from early stage to minimal volume of disease based on a new model of carcinogenesis. *Am J Obstet Gynecol.* 2008;198:351-356.
49. Fadare O, Khabele D. Molecular profiling of epithelial ovarian cancer. *My Cancer Genome.* http://www.mycancergenome.org/content/disease/ovarian-cancer. Accessed November 14, 2014.
50. Bast RC Jr, Mills GB. Dissecting "PI3Kness": the complexity of personalized therapy for ovarian cancer. *Cancer Discov.* 2012;2(1):16-18.
51. Westin SN, Herzog TJ, Coleman RL. Investigational agents in development for the treatment of ovarian cancer. *Invest New Drugs*. 2013;31(1):213-229
52. Gershenson DM. The life and times of low-grade serous carcinoma of the ovary. *Am Soc Clin Oncol Educ Book.* 2013. doi: 10.1200/EdBook_AM.2013.33.e195.
53. Ledermann J, Harter P, Gourley C, et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomized phase 2 trial [published online ahead of print May 31, 2014]. *Lancet Oncol.* 2014;15(8):852-861. doi: 10.1016/S1470-2045(14)70228-1.
54. Espinosa A, Gilbert J. Molecular profiling of thyroid cancer. *My Cancer Genome.* http://www.mycancergenome.org/content/disease/thyroid-cancer/. Accessed November 17, 2014.
55. American Cancer Society. Thyroid cancer. http://www.cancer.org/%20cancer/thyroidcancer/detailedguide/index. Accessed November 17, 2014.
56. Rusinek D, Szpak-Ulczok S, Jarzab B. Gene expression profile of human thyroid cancer in relation to its mutational status. *J Mol Endocrinol.* 2011;47(3):R91-R103.

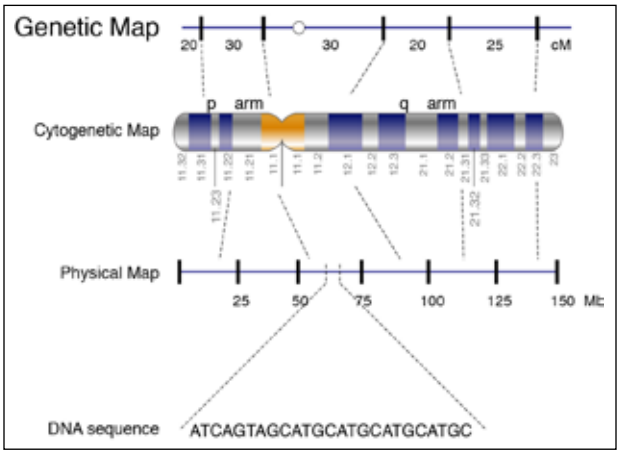
Emerging Genetic Tests and Companion Diagnostics in Oncology

The technical and scientific advances made in the field of genomic testing have resulted in the increasing proliferation of these tests in everyday clinical practice[1,2]. For oncologists and hematologists, genetic tests are used in screening, diagnosis, risk stratification, therapeutic management, and as a clinical decision-making tool in disease monitoring.

The rise in genetic testing also signals the end of the blockbuster drug era, ushering in the "niche-buster" era, defined as effective products delivered to identifiable patient segments. The result is a movement towards targeted therapies over the one-size-fits-all approach. The good news is that all cancer stakeholders—oncologists, payers, drug manufacturers, and of utmost importance, the patients—benefit from delivering the right drug to the right patient.

This more complete understanding of cancer biology has spurred the field of companion diagnostics (CDx), unleashing the potential for greater safety and efficacy, accelerated regulatory approval, and the possibility for value-based reimbursement.

A trend toward co-development of drugs and CDx stands in stark contrast to siloed development. Linking a molecular test to drug response requires a clinical trial. Ideally, co-development programs are initiated early on in trials, but tests are often an afterthought. This paradigm is changing as more co-development successes have become public, and the pressure continues to mount on pharmaceutical companies to replace brand name medications coming off patent protection.



A genetic map is a type of chromosome map that shows the relative locations of genes and other important features. The map is based on the idea of linkage, which means that the closer two genes are to each other on the chromosome, the greater the probability that they will be inherited together. By following inheritance patterns, the relative locations of genes along the chromosome are established.

With the success of targeted treatments such as Gleevec (imatinib mesylate) and Herceptin (trastuzumab) generating billion-dollar sales revenue, the switch to personally tailored medicine is a fait accompli. In this brave new world of personalized medicine, companion diagnostics have become the industry's "Rosetta Stone" and allow researchers to decode which patients will best respond to specific treatments, conduct smaller clinical trials, improve efficacy, reduce side effects, lower development costs, and streamline regulatory approval.

Since 2010, when the FDA approved the companion diagnostic HercepTest for the treatment of HER2+ breast cancer, there has been a groundswell of activity and regulatory support for the co-development of targeted therapies with companion diagnostics. Companion diagnostic testing predicts treatment response by identifying alterations in genes such as wild-type *KRAS* in colorectal cancer, anaplastic lymphoma kinase (*ALK*), in non-small-cell lung cancer (NSCLC), and the mutation in the *BRAF* gene associated with metastatic melanomas.

Regulatory agencies such as the FDA and European Medicines Agency (EMA) understand the profound implications that companion diagnostics will have in ensuring the safe and effective delivery of molecularly targeted agents. In draft guidance[3] issued on July 14, 2011, the FDA suggested that targeted oncology agents would only gain approval if reviewed in parallel with companion diagnostic tests, stating "the IVD [in vitro device] companion diagnostic device will be essential for the safe and effective use of the therapeutic product, and its use will be stipulated in the labeling of the therapeutic product."

In addition, the FDA states that, "for a novel therapeutic product, an IVD companion diagnostic device should be developed and approved or cleared contemporaneously to support the therapeutic product's safe and effective use."

Co-development is central to the paradigm shift occurring in the regulatory field of targeted medicines, the ramifications of which will soon be felt in the European Union. In a 2011 White Paper, the EMA signaled its intention of regulating companion diagnostics by keeping these genetic tests under the IVD directive and giving them a class C rating, which indicates a high individual risk and/or moderate public risk.

The FDA has demonstrated its willingness to approve agents that closely hew to the agency's draft guidance, as evidenced by the rapid approvals of both Zelboraf and Xalkori. However, the FDA has also shown its willingness

to reject targeted therapeutic oncologic agents because they lack a companion diagnostic. Such was the case in 2010, when the FDA rejected Omapro (omacetaxine mepesuccinate; ChemGenex) for the treatment of a subset of patients with leukemia with the *T315I* mutation.

All of this makes it essential to partner with a cutting-edge companion diagnostic company with the skills to smoothly navigate today's shifting regulatory terrain.

Targeted therapy is fast coming into focus. Companion diagnostics offer exceptional benefits for physicians, regulatory agencies, insurance providers, and most important, patients. Expanding the capabilities of what cancer trials can accomplish will undoubtedly result in higher quality healthcare that is extremely cost-effective. The increased clinical efficacy and fewer side effects associated with patient-stratified therapies may inevitably speed up approval times and lower clinical trial costs, making companion diagnostics a win-win for all involved.

REFERENCES

1. Khoury MJ, Dorman JS. The human genome epidemiology network. *Am J Epidemiol.* 1998;148:1-3.
2. Khoury MJ, Millikan R, Little J, Gwinn M. The emergence of epidemiology in the genomics age. *Int J Epidemiol.* 2004;33:936-944.
3. US Food and Drug Administration. Guidance for industry and food and drug administration staff - in vitro companion diagnostic devices. www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM262327.pdf. Accessed January 24, 2014.

Companion Diagnostic Companies

Qiagen

Qiagen's therascreenKRAS assay (colorectal cancer/Erbitux, Vectibix): Colorectal cancers overexpressing the epidermal growth factor receptor (*EGFR*) gene may respond well to Erbitux (cetuximab) and Vectibix (panitumumab), but not if the *KRAS* gene is mutated, common in colorectal cancers. The only FDA-approved KRAS test, this automated reverse-transcription polymerase chain reaction (RT-PCR) assay detects and quantifies 97% of all known *KRAS* mutations via fluorescent signals from labeled antibodies bound to mutant *KRAS* nucleic acids.

Qiagen's therascreenEGFR RGQ PCR Kit test (lung cancer/Gilotrif): Non-small-cell lung cancer (NSCLC) may respond well to the targeted therapy Gilotrif (afatinib), if the *L858R* mutation is present in the cells' *EGFR* gene. Quantitative RT-PCR detects and counts *L858R* nucleic acids via fluorescent signals from antibodies bound to them.

Qiagen Pipeline

Qiagen is developing a new, next-generation sequencing (NGS)-based test for the *SF3B1* gene mutations in blood cell cancers.

Qiagen and AstraZeneca are co-developing a plasma-sample liquid biopsy companion diagnostic assay for AstraZeneca's European Union-approved drug gefitinib (Iressa), potentially sparing patients with NSCLC outside the United States an invasive biopsy to qualify for Iressa therapy.

In October 2014, Qiagen filed for FDA premarket approval of a new drug-companion diagnostic combination, but has not yet named or described the drug. Qiagen has a similar agreement with Astellas Pharma for Astellas' EGFR inhibitor and its FGFR inhibitor, both now in Astellas' pipeline.

In mid-November 2014, Qiagen and Novartis began collaborating to develop companion diagnostic tests for current and future Novartis products. Qiagen has almost two dozen such collaborations now active with nine separate companies.

Qiagen's newest NGS system, GeneRead DNAseq V2, examines over 500 cancer-associated genes and lacks only the sequencer, still being developed, to be complete. Its panels now include the *SF3B1* gene. Several companies are working on drugs that target *SF3B1*, making a companion diagnostic test for the gene likely to be well received.

Dako

Dako's HER2 IQFISH pharmDx test (gastric cancer/Herceptin; breast cancer/Herceptin, Perjeta, Kadcyla): The *HER2* gene is amplified in up to 20% of breast cancers, stimulating tumor growth. In this RT-PCR test, green fluorescent DNA probes bind to HER2 DNA in the sample and red fluorescent probes bind to the gene's host chromosome 17. The ratio of green to red signals and absolute number of HER2 signals indicate the extent of *HER2* gene amplification.

The test is approved to identify patients with

breast cancer eligible for Herceptin (trastuzumab), Perjeta (pertuzumab), and Kadcyla (ado-trastuzumab emtansine), and patients with gastric cancer likely to respond to Herceptin.

The Dako HER2 CISH pharmDx (HER2/stage II breast cancer prognosis) is a color-coded immunohistochemistry (IHC) assay. DNA probes bind to HER2 or to chromosome 17. Bright-field microscopy is used to view the two targets and their contrasting color probes. The absolute number of *HER2* genes present and the ratio of *HER2* gene to chromosome 17 help determine whether *HER2* is amplified. The test is used to supplement other information currently used to help estimate prognosis in patients with stage II, node-positive breast cancer.

Dako C-KIT PharmDx (gastric cancer/Gleevec): This qualitative IHC test detects expression of *C-KIT* in tissue from patients diagnosed with gastrointestinal stromal tumor (GIST). Antibody probes bind to any *C-KIT* in tissue samples in Dako's Autostainer, with color contrast highlighting their presence, helping identify patients likely to benefit from Gleevec (imatinib/imatinib mesylate).

Dako Pipeline

In early 2014, Dako and Merck agreed to co-develop companion diagnostic tests for cancer drugs now in Merck's development pipeline, and later announced that the two companies would co-develop a companion diagnostic test using the cancer biomarker PD-L1. Dako also has collaborative companion diagnostic agreements with Bristol-Myers Squibb (BMS), Genentech, Eli Lilly, and Pfizer.

Ventana Medical Systems (Roche)

INFORM HER2 Dual ISH DNA Probe Cocktail (breast cancer/Herceptin) is used to help determine eligibility for Herceptin (trastuzumab). In situ hybridization (ISH) in Ventana's BenchMark XT autostainer, light microscopy, and NexES software quantify HER2 DNA probes and chromosome 17 probes bound to their respective targets, indicating the extent of any *HER2* amplification. Test results are considered with other factors to determine whether Herceptin is an appropriate treatment option. Unlike Ventana's similarly named test, Inform HER2/neu, it is not intended only for prognostic use, and is not limited to breast cancer.

Cobas EGFR Mutation Test (NSCLC/Tarceva): This test identifies patients with the L858R mutation in the epidermal growth factor receptor (*EGFR*) gene, indicating that the tyrosine kinase inhibitor Tarceva (erlotinib) may be efficacious. In this Real-time PCR test, DNA isolated from formalin-fixed paraffin-embedded (FFPE) slides is automatically amplified and analyzed on the Cobas 480Z analyzer. Results help to select patients with metastatic NSCLC for whom Tarceva is indicated.

Cobas 4800 BRAF V600 Mutation Test (melanoma/Zelboraf): The *V600E* mutation in the *BRAF* gene causes overexpression of the BRAF growth-stimulating protein, promoting proliferation in malignant cells harboring the mutation. About 90% of known *BRAF* mutations are *V600E* mutations. DNA from melanoma tissue is amplified and analyzed on the Cobas analyzer. Use in patients for whom the BRAF inhibitor drug Zelboraf (vemurafenib) is indicated.

Ventana Pipeline

In July 2014, Ventana and Merck announced collaboration to co-develop a companion diagnostic aimed at an unnamed target for use in multiple cancer types.

In October, 2014, Ventana announced collaboration with ImmunoGen to co-develop a companion diagnostic for ImmunoGen's IMGN853, targeting folate receptor alpha (FOL1), highly expressed in some cancers of the endometrium, ovary, and in some types of NSCLC, bringing Ventana's many companion diagnostic projects close to 180 in number, involving over 45 companies.

Abbott

Abbott's Vysis ALK Break Apart FISH Probe Kit test for ALK (lung cancer/Xalkori) One percent to 7% of NSCLC have mutations in the anaplastic lymphoma kinase (*ALK*) gene, stimulating tumor growth. Pfizer's targeted therapy Xalkori (crizotinib) is approved for late-stage NSCLC expressing mutant *ALK*. In this fluorescence in situ hybridization (FISH) assay, DNA probes for mutant *ALK* bind to any mutant *ALK* present and are quantified via automated counting of their fluorescent signals. Detection of *ALK* mutations indicates Xalkori as a treatment option. Vysis ALK is the only test for *ALK* mutations that is FDA-approved for clinical use.

Abbott's PATHVYSION HER-2 DNA Probe Kit (breast cancer/Herceptin): When the *HER2* gene is amplified, it stimulates tumor growth. Herceptin inhibits *HER2* activity. DNA probes with contrasting colors bind to copies of

either *HER2* genes or to copies of its host, chromosome 17, in FFPE breast cancer tissue. The absolute number of *HER2* genes and the ratio of *HER2* to chromosome 17 stratify extent of any *HER2* amplification. The assay is also used to predict disease-free survival and overall survival in stage II, node-positive breast cancer treated with some chemotherapy regimens. PathVysion was the first gene-based test approved for identifying patients eligible for a specific cancer treatment (2001).

Abbott Pipeline

In June 2014, Abbott and the molecular diagnostics company Biocartis announced collaborative efforts to develop companion diagnostic tests. Abbott currently is evaluating dozens of molecular targets in over 20 different cancers for possible companion diagnostic tests. These include real-time PCR-based assays for *MAGEA3* in melanoma and NSCLC; *PRAME* in NSCLC; and *KRAS* and *BRAF* in colon cancer, lung cancer, and melanoma. Abbott plans to develop a sequencing-based companion diagnostic test for *c-KIT, GNAQ,* and *GNA11* aberrations in melanoma.

BioGenex

BioGenix's InSite HER2/neu Mouse Monoclonal Antibody (Clone C1B11) Kit (breast cancer/ Herceptin): When the *HER2* gene is amplified, it promotes malignant cell proliferation and tumor growth. Herceptin inhibits *HER2* activity. BioGenex's InSite HER2 assay is an IHC test used to identify patients likely to benefit from treatment with Herceptin because their tumor cells have abnormally high levels of the *HER2/neu* gene product, from amplification and/or overexpression of *HER2*. Antibody probes for *HER2* are introduced to tissue from FFPE tissue sections. Stains visually isolate *HER2,* and the samples are examined with light microscopy to determine whether the gene is amplified. BioGenix notes that the test should not be used alone to indicate Herceptin use, but should be used in conjunction with morphological tissue analysis, and should be evaluated by a qualified pathologist within the context of patient clinical history and other diagnostic results.

BioGenex Pipeline

BioGenex and Abbott have long had a collaborative agreement in which Abbott uses BioGenex's Xmatrx system, capable of running IHC, ISH, chromogenic in situ hybridization (CISH), FISH, microRNA (miRNA), and ISH PCR assays, to help develop its companion diagnostic tests, among other purposes. In 2009, the companies signed an agreement to extend this collaboration, with a focus on co-marketing BioGenex's Xmatrx system for use in conjunction with present and future automated FISH tests developed by Abbott. The extended agreement allows Abbott customers the option of running Abbott's IHC- and ISH-platform tests on BioGenex's Xmatrx system, and allows BioGenex customers to run Abbott FISH tests on the Xmatrx. Under the terms of the co-exclusive agreement, BioGenex's Xmatrx used for some Abbott tests is co-branded, and Abbott guarantees BioGenex minimum sales volumes guarantees over several years, as the two companies expect to increasingly use the arrangement in co-developing companion diagnostic tests.

Life Technologies

Life Technologies' SPOT-Light HER2 CISH Kit (breast cancer/ Herceptin): The *HER2* gene, when amplified in breast cancer cells, as it is in up to 20% of breast cancers, promotes aggressive tumor growth. Herceptin (trastuzumab) inhibits activity of HER2. It is approved for use along with several specific chemotherapy regimens or as a monotherapy after several different chemotherapy drugs.

Life Technologies says that the assay, FDA-approved in 2008, was developed to assess *HER2* amplification through a process more practical for most standard histopathologic labs than the more complex FISH assay. It can be used with FFPE or fresh tissue. The assay's CISH process uses DNA probes with affinity for HER2 to bind to *HER2* genes in the sample. Color stains visually isolate the probe-gene complexes, counted using standard bright-field microscopy. The test is used along with other clinical and pathologic data used to judge suitability of treating an individual patient's breast cancer with Herceptin.

Life Technologies Pipeline

In 2012, Life Technologies began a collaborative companion diagnostics program with BMS to develop tests for current and pipeline BMS cancer drugs.

Life Technologies has a collaborative agreement, also begun in 2012, with CollabRx, through which the two companies expect that CollabRx's wide gathering of clinical and genomics-based data will provide insights improving Life Technologies' ability to develop companion

diagnostic tests for cancer. Life Technologies has a collaborative agreement with Merck, begun in 2013, to co-develop companion diagnostic tests for current and future Merck drugs, but neither company has yet said which Merck drugs constitute the focus of the program.

In 2013, Life Technologies completed a round of acquisitions (Pinpoint Genomics, Navigenics, and Compendia) that the company says will speed its work in biomarker discovery, making Life Technologies a prime potential partner for pharma companies developing companion diagnostics in cancer.

Leica Biosystems

Leica Biosystem's Bond Oracle Her2 IHC test (breast cancer/Herceptin): This assay is used to help select patients likely to respond well to treatment with the anti-HER2 monoclonal antibody Herceptin. The *HER2* gene, when present in normal quantities in a cell, helps regulate cell growth and division. However, in up to 20% of breast cancers, the gene is amplified, and its overabundant product acts to stimulate cell proliferation and tumor growth. Herceptin inhibits the activity of HER2. This test is FDA-approved as an aid in determining a patient's eligibility for treatment with Herceptin. The test is a semi-quantitative IHC assay in which HER2 antibody probes bind to any HER2 DNA in FFPE tissue samples. Leica's Bond-Max automated slide staining instrument stains the HER2-probe complexes, which are then counted and compared to the number of copies of chromosome 17 in the samples. The absolute number of *HER2* genes and the ratio of *HER2* to chromosome 17 indicate the extent of any *HER2* amplification. The system has been shown in studies to have high concordance with other systems for detection of *HER2* amplification.

Leica Biosystems Pipeline

In August 2014, Leica Biosystems announced an agreement to collaborate with BMS to develop companion diagnostic tests to help identify patients who can benefit from therapies for cancer and some other diseases. Leica indicated these new companion diagnostic tests would be IHC tests meant to be performed on Leica's Bond system.

In March 2013, Leica and Synthon Biopharmaceuticals unveiled an agreement to collaborate on development of a companion diagnostic test meant to identify patients who can most benefit from one of Synthon's targeted therapies in development, an antibody-drug conjugate (ADC) for the treatment of some solid tumors. The agreement also provides for additional work to develop additional companion diagnostic test/drug combinations.

bioMérieux

bioMérieux THxID BRAF kit (melanoma/Tafinlar or Mekinist): The *BRAF* gene is a proto-oncogene that is helpful in normal cell processes when undamaged, but oncogenic when mutated in specific ways, leading to constitutive growth signaling that promotes proliferation and inhibits apoptosis. Two *BRAF* mutations, the *BRAF V600E* and *BRAF V600K*, activate the gene's oncogenic signaling. Approximately 85% of all *BRAF* mutations in melanoma are *BRAF V600E* mutations, with another 10% being *V600K* mutations. In each mutation, a single substitution of one nucleotide for another results in activating the oncogenic functions of the gene. In patients with either of these mutations, two inhibitors of those mutations can produce superior outcomes to traditional chemotherapy and/or immunotherapy regimens. The company's THxID BRAF kit is used to help identify patients whose melanoma cells have the *V600E* mutation, making them likely to respond to Tafinlar (dabrafenib) and patients whose melanoma cells carry either the *V600E* or the *V600K* mutation, making their cancers likely to respond to Mekinist (trametinib). The test is a RT-PCR test performed on the ABI 7500 Fast Dx system.

bioMérieux Pipeline

The company is currently collaborating with Ipsen to develop a companion diagnostic test for an Ipsen breast cancer drug currently in the early stages of clinical development. As a part of the agreement, bioMérieux will design a test to identify patients with breast cancer likely to benefit from the new treatment. The assay is intended both to help Ipsen with clinical development of its drug and to later be used as a companion diagnostic test, with possible future commercialization by bioMérieux. The company also has collaborative projects with GlaxoSmithKline to co-develop two companion diagnostic tests: one for use in breast cancer and the other for melanoma. The company currently also collaborates with third-party payers for patient care. In addition to its tests used only for companion diagnostics, the company's subsidiary bioTheranostics has two assays for related purposes in cancer: the CancerTYPE ID test helps to identify the origin of cancers of unknown

origin that have metastasized, and the Breast Cancer Index is marketed to aid in predicting risk of breast cancer recurrence.

Myriad Genetics, Inc.

BRACAnalysis CDx (ovarian cancer/Lynparza): Results of the test help identify patients with ovarian cancer who harbor a deleterious or suspected deleterious germline *BRCA* variant, which indicates that the patient may benefit from treatment with the PARP inhibitor Lynparza (olaparib). The efficacy of the test and the drug were demonstrated in clinical trials, leading to an accelerated FDA approval.

BRACAnalysis CDx is the only FDA approved test for use in conjunction with Lynparza. The novel companion diagnostic looks specifically at variants in the protein coding regions and intron/exon boundaries of the *BRCA1* and *BRCA2* genes using genomic DNA obtained from whole blood specimens collected in ethylenediaminetetraacetic acid. PCR and Sanger NGS identify single nucleotide variants and small insertions and deletions. Large deletions and duplications in BRCA1 and BRCA2 are detected using multiplex PCR.

Myriad Genetics, Inc. Pipeline

Myriad has a deep diagnostic pipeline across the disease spectrum, including clinical tests in areas of unmet medical need. In the short-term, several tests are expected to gain approvals and become available. The company maintains several partnerships around its BRACAnalysis CDx test with companies exploring PARP inhibitors. Additionally, research collaborations are underway for the company's myChoice HRD test, which detects DNA repair deficiencies.

Movement on Reimbursement, Regulatory Fronts

The year 2015 promises to be busy one for those in the molecular diagnostics industry, as January brought a string of important developments on from regulators, from a key Medicare contractor, and even from President Obama.

On January 8-9, 2015, FDA hosted a workshop to outline how it will go about regulating laboratory-developed tests (LDTs) from an estimated 11,000 laboratories and academic institutions.[1] To supporters, this a logical and necessary step to ensure patient safety. To detractors, it's an unnecessary overreach that will drive smaller testing labs out of business, causing industry consolidation at the expense of innovation and consumers.

Until now, LDTs have been governed by CMS under the Clinical Laboratory Improvement Act (CLIA) of 1988; as a practical matter, very few tests are cleared by FDA before they hit the market. As testing soared, especially in cancer care, payers complained that CMS billing codes meant no one understood what they were buying. In recent years, payers began to raise evidence bar before they would fund tests; because the industry lacked the same clinical trial outline that therapeutics have at FDA, many tests hit sudden reimbursement roadblocks.

Then, on January 22, 2015, Palmetto GBA, the Medicare administrative contractor the Carolinas, Virginia and West Virginia, posted a notice outlining requirements for reimbursement of somatic comprehensive genomic profiling for certain patients with non-small cell lung cancer.[2] This came without warning 2 days after the president had called for a precision medicine initiative in his State of the Union address; while the items were not expressly linked, *The New York Times* did so in its coverage, and quoted longtime industry expert Bruce Quinn, MD, PhD, MBA, who called Palmetto's decision a "watershed event."[3]

CMS has the right, by law, to use Palmetto's unique MolDx program as a benchmark for the rest of Medicare, although there's been no word if that will happen. Eight days later, however, FDA Commissioner Margaret A. Hamburg, MD, issued a blog post that suggested FDA will apply "practical regulation" to next-generation sequencing (NGS), rather than data on individual genetic variants.[4] Hamburg, too, linked FDA's position to the precision medicine initiative, which seeks $215 million for a package of research efforts and regulatory efforts, including those at FDA.

At Long Last, FDA Steps In

Pharmaceutical companies and payers, for different reasons, have long sought to bring some order to regulatory issues in molecular diagnostics industry, and FDA moved to do on July 31, 2014. So far, medical establishment titans stand on opposite sides in this battle. The next phase will begin after February 2, 2015, when the FDA closes the comment period on its September 30, 2014, draft guidance for regulating LDTs.5 The FDA has committed to issuing final rules this year, although many signs indicate that the process will be slowed down by administrative challenges or even a lawsuit.

FDA moved to exercise regulatory authority over diagnostic tests, which are increasingly important in cancer care, as the rise of genomic medicine has allowed clinicians to tailor cutting-edge (and very expensive) treatments to patients based on their genetic characteristics.[6] Both the American Society of Clinical Oncology (ASCO) and the American Cancer Society Cancer Action Network support the FDA's action, citing a need for a stronger evidence base in diagnostics.[5]

Opposing the FDA's move are the American Medical Association (AMA) and the Association for Molecular Pathology (AMP), along with the testing industry's trade group, the American Clinical Laboratory Association (ACLA).[7-9] In a statement accompanying its white paper, the AMP pointed at the twin forces that threaten to shake out the testing industry: the prospect of increased regulation, and the ongoing challenge of winning reimbursement from Medicare.[8]

"The FDA's new policies will effectively reformulate existing medical device regulations and consider medical professionals as manufacturers which will impose substantially new and duplicative requirements on clinical laboratories and hospitals," the AMP statement said. "Meanwhile, CMS, who runs Medicare, the nation's largest insurer and whose actions are frequently mimicked in the private sector, has taken a heavy handed approach in denying coverage or reducing payment for several medically necessary molecular pathology tests.

"Unfortunately, health care providers—those developing and delivering innovative diagnostic tests—along with patients, who are the ultimate intended beneficiaries, are caught in the middle," AMP said.[8]

Support for FDA Regulation

Arguments supporting and opposing FDA oversight were best outlined in a pair of commentaries published January 5, 2015, by *JAMA Oncology*.[5,7] The dueling viewpoints appeared days before the FDA workshop on the regulatory process.[1]

As discussed by Joshua Sharfstein, MD, the FDA's decision is justified for several reasons:

- There have been some high-profile examples of faulty tests, including an April 2014 failure that required a warning in CDC's *Mortality and Morbidity Weekly Report*. The lack of FDA oversight raises the specter of cancer patients receiving the wrong therapy or false positives that lead to unnecessary treatment.
- The FDA has no authority to require reporting of adverse events; thus, thousands of patients may be affected or harmed before a problem comes to light. Sharfstein cited a case involving a suspect test for directing statin therapy that was given to 150,000 patients before flaws were uncovered.[5]
- Lack of regulation results in a lack of evidence to guide clinicians.[5] Disagreements over standards of evidence have also created unpredictability and unevenness of reimbursement criteria among insurers, who must decide whether to pay for the tests. Since 2011, the emerging bar of "clinical utility" has frustrated many test manufacturers; some argue that CMS' chief Medicare contractor in this realm, Palmetto GBA, makes impractical demands regarding evidence for reimbursement.[10]
- Sharfstein asserts that proposed carve-outs for tests for rare diseases, for which no other LDT is available, and plans to phase in regulations will address worries about patient access to tests in the short term.[5]

ASCO provided high-profile support for the FDA's effort with comments submitted to the House Committee on Energy and Commerce, Health Subcommittee, which had released a white paper on creating a new regulatory framework for the tests December 9, 2014.[11]

"In contemporary oncology practice, a patient's treatment options are increasingly driven by detection of molecular abnormalities in the tumor that drive treatment selection," ASCO president Peter P. Yu, MD, FASCO, said in a letter to the subcommittee. "ASCO believes that the tests used to detect those abnormalities must be of the highest quality and thoroughly validated before being offered to doctors and patients. Our patients depend on high quality tests as much as they depend on carefully studied, safe and effective drugs to achieve the best possible outcomes." Also, ASCO sent Cancer Research Committee Chair Edward Kim, MD, to participate in the FDA workshop.[11]

Arguments Against FDA Oversight

The case against regulation, made in *JAMA Oncology* by James P. Evans, MD, PhD, and Michael S. Watson, PhD, is summed up this way: The testing industry includes many small hospital and academic research labs, which are responsible for much of the innovation of recent years but would lack the financial resources to meet the bar of FDA regulation. Forcing these groups to do so would stifle progress in genomic medicine, which would harm the very consumers that regulators say they want to help.[7]

AMA's early statement on FDA oversight, which came the day after the July 31, 2014, announcement, echoed this concern. The AMA "believes that laboratory developed testing (LDT) services offer patients access to safe and high quality diagnostic services that are essential to patient care," said Barbara L. McAneny, MD, chair of the AMA board. "This proposal adds an additional layer of regulatory requirements which may result in patients losing access to timely life-saving diagnostic services and hinder advancements in the practice of medicine."[12]

Evans and Watson point to the example of tests for BRCA1 and BRCA2, which were only available through Myriad Genetics until the US Supreme Court largely unraveled the company's monopoly in 2013. Labs rushed into the market, and the results have given patients choices and forced down the price of the tests themselves. Adding FDA approval on top of existing CLIA requirements would reconsolidate the industry, Evans and Watson argue.

They acknowledge, however, that the genetic-based tests that direct cancer care are more complex than the vast majority of LDTs that could come under the FDA's umbrella. Their solution is to update the CLIA with requirements specific to genetic tests, not add a new regulatory layer.[7]

Finally, Evans and Watson question whether FDA has the authority to regulate tests as "medical devices." Rather, they write, tests are "procedures" that depend not only on technology but also on the expertise of the laboratory, and this will be a poor fit for FDA-style rulemaking. They predict, as others do, that "Given the questionable legitimacy of the FDA's proposed action, if these changes are implemented they will most certainly trigger expensive and time-consuming legal challenges." Such challenges would most likely come from the ACLA, according to press accounts.[9]

ACLA Responds to FDA

The testing industry appears ready to push back hard against

National Human Genome Research Institute researcher uses a pipette to prepare DNA for sequencing.

the FDA. ACLA released its own white paper January 7, 2015, the day before the agency's workshop, which it described as a "systematic and detailed rejection of the (FDA) proposal." The document was developed with former solicitor general Paul D. Clement and Harvard constitutional law expert Laurence H. Tribe, and appears to outline arguments that would be used in administrative or legal challenges against the FDA's attempt to regulate the industry.[13]

As expected, a chief argument involves the FDA's classification of tests as "medical devices." Other arguments raised by the ACLA include:

- FDA regulation of LDT interferes with the practice of medicine.
- The ACLA asserts that the FDA's use of guidance documents bypasses the Administrative Procedure Act (APA), which outlines the process that agencies must use in crafting regulations.

While this last item might seem like a procedural argument, the ACLA indicates that it is critical: failure to oppose the FDA's use of the guidance document at this stage would lay the groundwork for regulation of the industry outside APA's scope going forward. However, there is some irony to the ACLA arguments in the white paper: it cites CMS' long-standing ability to regulate the testing market through CLIA, despite the fact that test makers in recent years have spent plenty of time complaining about CMS reimbursement.

"In the interest of patients and their continued access to critical laboratory testing services, it is incumbent upon the FDA to reverse course and withdraw its proposed agency overreach," said Steve Rusckowski, chairman of the ACLA Board of Directors and CEO of Quest Diagnostics.[13]

What Palmetto Seeks to Do

As discussed in *The Cancer Letter*, Palmetto's directive applies only to its jurisdiction for now. The decision is limited to tests for patients who have either never smoked or were light smokers who quit (equal to or less than 15 pack years) and tested negative for EGFR mutations and EML-ALK rearrangements. This will allow patients to be treated with a targeted therapy for which they would have been otherwise ineligible.[2]

Experts who spoke with *The Cancer Letter*, as well as Quinn and Rina Wolf, MHA, a vice president for XIFIN, told attendees at Patient-Centered Oncology Care, a meeting of *The American Journal of Managed Care*, that comprehensive sequencing technologies could render moot current challenges that arise when tissue samples are depleted before all necessary tests are run.[2,14]

REFERENCES

1. Public workshop: framework for regulatory oversight of laboratory developed tests (LDTs), January 8-9, 2015. FDA website. http://www.fda.gov/MedicalDevices/NewsEvents/WorkshopsConferences/ucm423537.htm. Accessed January 8, 2015.
2. Goldberg P. CMS opens door to coverage of comprehensive genomic sequencing. *The Cancer Letter. 2015;41(3):1-6.*
3. *Pear R. Obama to request research funding for treatments tailored to patients' DNA. The New York Times website.* http://www.nytimes.com/2015/01/25/us/obama-to-request-research-funding-for-treatments-tailored-to-patients-dna.html?_r=0. Published January 24, 2015. Accessed February 2, 2015.
4. Hamburg MA. FDA considering how to tailor its oversight for next generation sequencing. FDA Voice. http://blogs.fda.gov/fdavoice/index.php/2015/01/. Published January 30, 2015. Accessed February 3, 2015.
5. Sharfstein J. FDA regulation of laboratory-developed diagnostic tests: protect the public, advance the science [published online January 5, 2015]. *JAMA Oncology.* doi:10.1001/jama.2014.18135.
6 Caffrey MK. FDA moves to regulate thousands of regulatory tests. *Am J Manag Care. 2014;20(SP11):SP338.*
7. *Evans JP, Watson MS. Genetic testing and FDA regulation: overregulation threatens the emergence of genomic medicine [published online January 5, 2015]. JAMA Oncology.* doi:10.1001/jama.2014.18145.
8. AMP releases "molecular diagnostic perfect storm" paper addressing the convergence of regulatory and reimbursement forces that threaten patient care [press release]. http://www.amp.org/documents/20150108_AMPReleasesPerfectStormPaper.pdf. Bethesda, MD: Association for Molecular Pathology; January 8, 2015.
9. Saxena V. FDA says expedited access PMA, new LDT regulations to be implemented this year. Fierce Medical Devices website. http://www.fiercemedicaldevices.com/story/fda-says-expedited-access-pma-new-ldt-regulations-will-be-implemented-year/2015-01-09. Published January 9 2015. Accessed January 18, 2015.
10. Caffrey MK. When science outpaces the payers: reimbursement in molecular diagnostics. *Am J Manag Care. 2014;20(SP7):SP234-SP236.*
11. *ASCO submits comments to Congress on FDA's proposed regulation of laboratory developed tests [press release]. American Society of Clinical Oncology website.* http://www.asco.org/advocacy/asco-submits-comments-congress-fda%E2%80%99s-proposed-regulation-laboratory-developed-tests. Alexandria, VA: American Society of Clinical Oncology; January 5, 2015.
12. AMA statement on FDA proposal regarding diagnostic testing [press release]. American Medical Association website. http://www.ama-assn.org/ama/pub/news/news/2014/2014-08-01-fda-proposal-diagnostic-testing.page. Washington, DC: American Medical Association; August 1, 2014.
13. ACLA releases white paper detailing legal arguments against FDA's proposal to regulate laboratory developed tests (LDTs) as "medical devices" [press release]. http://www.prnewswire.com/news-releases/acla-releases-white-paper-detailing-legal-arguments-against-fdas-proposal-to-regulate-laboratory-developed-tests-ldts-as-medical-devices-300017177.html. Washington, DC: PRNewswire; January 7, 2015.
14. *The American Journal of Managed Care. The Road to Personalized Medicine: Clinical Utility and Reimbursement in Molecular Diagnostics. Patient-Centered Oncology Care 2014 website.* http://www.ajmc.com/ajmc-tv/live/pcoc14/Panel4. Produced November 14, 2014. Accessed February 3, 2014.

Lab-Based Gene Testing Companies

Genomic Health

Genomic Health Oncotype DX Breast Cancer Assay: In this prognostic test for patients with stage I or stage II, estrogen receptor-positive (ER+), HER-2 negative breast cancer, reverse-transcription polymerase chain reaction (RT-PCR) amplifies nucleic acids produced by genes associated with breast cancer disease processes, response to treatment, and prognosis, Software and proprietary algorithms quantify probability of both distant recurrence and benefit from chemotherapy.

Genomic Health Oncotype DX Breast Cancer Test for DCIS scores the risk of local recurrence—either ductal carcinoma in situ (DCIS) or carcinoma—in patients with DCIS after local excision, whether adjuvant tamoxifen is used or not. RT-PCR amplifies RNA from genes key to progression of DCIS and breast cancer. Quantification and proprietary algorithms produce the numerical score.

The Oncotype DX Colon Cancer Assay predicts the probability of recurrence of stage II and stage III colon cancer. High throughput, parallel RE-PCR (TaqMan RT-PCR) amplifies and quantifies nucleic acids from key genes in pathways associated with colon cancer progression, response to treatment, and prognosis. A proprietary formula factors in the genes' roles and importance in the disease to produce a risk recurrence score between 1 and 100.

The Oncotype DX Prostate Cancer Assay predicts aggressiveness in early-stage, localized prostate cancer. RT-PCR detects and quantifies the expression of 17 genes in four pathways correlated with prostate cancer aggressiveness, producing a Genomic Prostate Score (GPS). The test can be performed on needle biopsy tissue.

Agendia

Agendia's MammaPrint quantifies likelihood of metastasis in stage I or II node-negative breast cancer with tumors <5 cm. A DNA microarray gauges expression of 70 genes associated with breast cancer prognosis. Algorithms translate expression levels into a quantified risk.

Agendia's Blueprint test is an 80-gene, DNA microarray test used with Agendia's MammaPrint to identify a patient's molecular subtype of breast cancer (luminal A, luminal B, basal-type, or HER2-positive), indicating the patient's likely response to some therapies before or after surgery.

Agendia's Targetprint is a DNA microarray test that identifies patients likely to benefit from hormonal or (HER2) targeted therapies.

Agendia's Coloprint stratifies risk of distant recurrence in stage II and III colon cancer. A DNA microarray quantifies expression of 7 genes associated with colon cancer aggressiveness and 5 reference genes and proprietary algorithms to classify the cancers as high risk or low risk (no intermediate classification).

Agendia's Theraprint test (for breast cancer), used to help guide therapy, uses a DNA microarray quantifying 55 biomarkers and variants in 4 potential biomarker genes to predict response to various hormonal, biological, and chemotherapeutic therapies. Some biomarkers correlate with response or resistance, others with specific drugs or drug classes. The report details clinical relevance of each biomarker.

Agendia's Theraprint test (for colon cancer) is used to help guide therapy. A DNA microarray measures 39 biomarkers and 4 potential biomarker genes correlated with response or resistance to various specific hormonal, biological, and chemotherapeutic drugs and drug classes.

Agendia's Symphony Genomic Profile (breast cancer) merges results from MammaPrint, Blueprint, and Targetprint (see above) to help guide therapeutic decision making.

Single Gene Mutation Tests: Agendia performs real-time PCR mutation analyses on 4 genes (*EGFR, KRAS, BRAF,* and *PIK3CA*) that are key in determining eligibility for specific targeted therapies.

NanoString

NanoString's Prosigna Breast Cancer Prognostic Gene Signature Assay is a multigene assay used as a prognostic indicator in women who have previously undergone standard-of-care surgical treatment for locoregional breast carcinoma. It is indicated, when used along with other clinical and pathologic information, to quantify the probability of 10-year, distant recurrence-free survival in 2 categories of postmenopausal patients with hormone receptor–positive (HR+) breast cancer: (1) patients with lymph node-negative, stage I or II breast cancer expected to be treated with adjuvant endocrine therapy alone; and (2) patients with lymph node-positive (1 to 3 nodes) stage II breast cancer expected to be treated with adjuvant endocrine therapy alone.

Based on the 50-gene PAM50 gene expression signature, the test report provides a numerical risk-of-recurrence (ROR) score from 0 to 100; a risk category; and identification of cancer subtype: luminal A or B; HER2-enriched; or basal-like.

Fluorescent mRNA probes hybridize with gene-specific mRNA that is unique to any of the 50 genes in the PAM50 gene signature. NanoString's automated nCounter counts the color-coded transcript-probe complexes, generating a profile of gene expression levels. That profile is compared with the 4 reference profiles of the PAM50 gene signature. A proprietary algorithm combines the extent of similarity with the PAM50 reference profiles, a proliferation score, and tumor size, to produce the individual's 0-to-100 Prosigna ROR score.

Vysis (Abbott)

UroVysion Bladder Cancer Kit: Aneuploidy of chromosomes 3, 7, and 17 with loss of the 9p21 locus occurs in most bladder carcinomas. Probes target those chromosomes and locus 9p21. This fluorescence in situ hybridization (FISH)-based test is used adjunctively in diagnosis and monitoring of bladder carcinoma.

RealTime mS9 Colorectal Cancer Assay: The Septin 9 gene (*SEPT9*) is involved in cell division and may be a tumor suppressor gene. Methylation of *SEPT9* is associated with colorectal cancer. This real-time, two-probe PCR-based test detects methylated *SEPT9* in plasma. It may be useful to detect colorectal cancer when confirmed by colonoscopy.

CLL FISH Probe Kit (B-cell CLL): This FISH-based diagnostic/prognostic test quantifies presence of chromosomal aberrations associated with B-cell chronic lymphocytic leukemia (CLL) to diagnose and subtype CLL and adjunctively to help determine prognosis.

CEP 12 DNA Probe Kit (B-cell CLL): Used conjunctively with other clinical and pathologic features to help determine prognosis, this DNA probe FISH test detects the trisomy 12 aberration associated with poor outcome.

EGR1 FISH Probes Kit (AML): This FISH-based test for deletion of the early growth response-1 (*EGR1*) gene is used conjunctively with other clinical and pathologic information as a prognostic test in acute myeloid leukemia (AML).

D7S486/CEP7 FISH Probe Kit (AML and MDS): In this prognostic test, FISH detects aberrations in chromosome 7 associated with poor outcomes in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS).

Melanoma FISH Probe Kit: This DNA probe-based FISH test is used to help diagnose melanoma. It quantifies aberrations in melanoma-associated genes (*RREB1, MYB*, and *CCND1*) that can help identify melanoma.

Hologic

Hologic

Progensa PCA3 Assay is an mRNA-based test to help judge the need for a repeat prostate biopsy in men who earlier had a negative biopsy, but who have clinical or pathologic indicators suggestive of prostate

cancer. It can help lessen the number of unnecessary prostate biopsies. It can also be used to help monitor prostate cancer.

The prostate cancer antigen-3 (*PCA3*) gene, expressed only in prostate tissue, is highly overexpressed in prostate cancer. RNA from the *PCA3* gene and from the prostate-specific antigen (PSA) are extracted from urine and amplified for counting. The ratio of PCA3 RNA relative to PSA RNA is used to determine a PCA3 score, with higher scores indicating a greater likelihood of prostate cancer. Used in conjunction with other clinical and pathologic factors to determine need for a repeat biopsy.

The PSA3 score can also be used to help judge the aggressiveness of the cancer, with a higher score indicating a higher likelihood that the cancer is aggressive.

Hologic's Aptima HPV Assay detects overexpression of mRNA from *E6* and/or *E7* genes to detect 14 high-risk strains of human papillomavirus (HPV) that cause nearly all cervical cancers. It is run on Hologic's automated systems, using the proprietary ThinPrep sample procedure.

Hologic's Cervista HPV HR detects all 14 oncogenic strains of HPV. DNA probes bind to HPV DNA sequences, releasing detectable amplified florescent signals when cleaved by proprietary enzymes in a series of reactions dependent on the presence of strain-specific HPV DNA.

Hologic's ThinPrep Pap Test is a liquid-sample based procedure. Tissue is collected and preserved in a liquid solution. Slides are prepared by Hologic's proprietary ThinPrep automated stainer and examined by microscopy to detect abnormal or diseased cells. A proprietary computer imaging system can be used prior to manual microscopy to suggest slide areas most important for review.

Myriad Genetrics

Hereditary Cancer Risk Assays

myRisk assesses risk of developing hereditary breast, ovarian, gastric, colorectal, pancreatic, melanoma, prostate, or endometrial cancers from germline mutations in 25 cancer-associated genes. Next-generation sequencing of DNA from blood or mouthwash helps stratify risk.

BRACAnalysis: Risk of hereditary breast or ovarian cancer due to *BRCA1/2* mutations is assessed by PCR and microarray comparative genomic hybridization (CGH); analysis is updated continually over the patient's lifetime.

COLARIS: DNA from blood analyzed by quantitative PCR (qPCR) detects germline mutations in 5 genes associated with hereditary colon, uterine, or endometrial cancers. Proprietary software automates risk analysis.

COLARIS AP gauges the risk of hereditary colorectal polyps and colorectal cancer due to germline mutations in the *APC* and *MYH* genes. Sequencing of DNA from a blood sample is combined with microarray-CGH based examination of any rearrangements.

Melaris assesses risk of developing hereditary melanoma. DNA from blood is amplified by PCR, then directly sequenced. Computer and visual inspections detect germline mutations in the *P16* gene.

Panexia gauges risk of developing hereditary pancreatic cancer. Blood or mouthwash DNA is amplified by PCR and sequenced directly. Computer analysis, visual confirmation and/or microarray-CGH detect mutations in hereditary pancreatic cancer-associated genes *PALB2* and *BRCA2*.

Sporadic Cancers

myPlan for lung cancer measures aggressiveness in early-stage non-small-cell lung cancer (NSCLC). Tissue RNA is examined by qPCR, quantifying expression of 31 genes important in NSCLC, then combined with stage to score aggressiveness.

myPath Melanoma uses qRT-PCR and algorithmic analysis of 23 melanoma-associated genes to generate a score used with other clinical and pathologic information to help diagnose sporadic melanoma.

Prolaris uses RT-PCR-amplified mRNA to quantify expression of 46 genes, generating a cell-cycle progression score, indicating aggressiveness of a patient's prostate cancer to aid in prognosis.

Next-Generation Sequencing: *Why It Matters for Cancer Care*

Next-generation sequencing is a collection of novel methods for determining the sequence of genes. These techniques drive down the cost of genetic testing in cancer and enable screening for multiple genes at once.

In 1975, Fred Sanger published a seminal paper on a novel method for sequencing DNA–a method that is now known as Sanger sequencing.[1] Sanger's method involves incubating samples of DNA in test tubes rich in the individual bases (adenine, thymine, cytosine, and guanine) that form the sequence of DNA.[2]

Normally, these four bases in the presence of heat, enzymes, and primer strands would enable DNA to replicate as it does in nature. Sanger modified the normal process of replication by introducing a radioactively labeled base lacking two hydroxyl groups that are necessary for the base to pair with its complement on the original DNA strand. As a result, rather than replicating normally, a series of strands of varying length form, and the length of each strand corresponds to a location where a strand-terminating base stopped DNA replication from proceeding.[2]

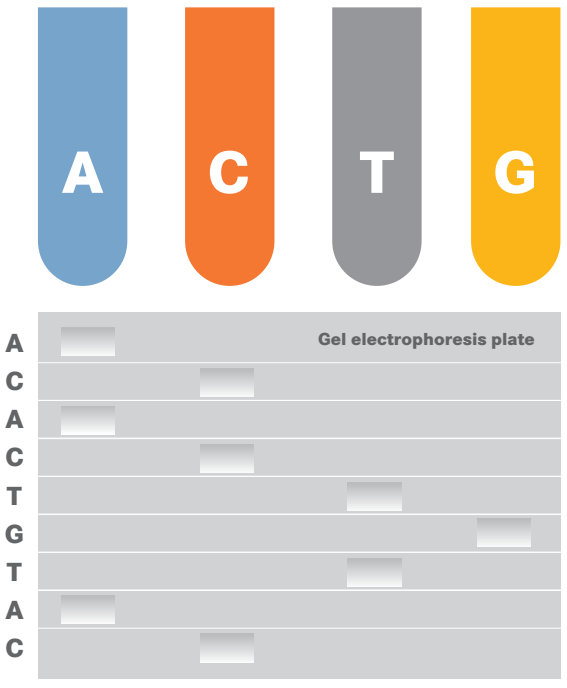
By repeating this experiment in four test tubes, each containing a different strand-terminating base, a series of small strands of DNA form, the length of which correspond with the position of a base. Using gel electrophoresis to measure the size of each strand, Sanger determined the sequence of base pairs that made up the DNA strand under consideration (**Figure 1**).[2] Using a modified form of this method, by 1987, Applied Biosystems had created the first automatic sequencing machine, which was able to read 500,000 bases each day–a number that had increased to 2.9 million bases daily by 2012.[3]

Sanger sequencing is very reliable, but it is also cumbersome, laborious, and expensive.[4] Although the cost of sequencing dropped steadily over time, the essential chemistry behind sequencing–use of strand-terminating bases–remained unchanged until 2008, when a new set of technologies collectively known as next-generation sequencing (NGS) began reducing the cost of sequencing at an even more dramatic rate.[5]

With NGS, price reductions that had previously taken years occurred in a matter of months. In the 6 years from September 2001 to January 2008, the cost of sequencing a human genome dropped by more than 90% from $95.3 million to $3.0 million, but over the 10 months from January to October 2008, the cost of sequencing a genome had already fallen from $3.0 million to $342,502. The trend shows no sign of slowing. As of July 2014, the most recent available data from the human genome project place the cost of sequencing an entire human genome at $4905.[5]

The NGS techniques that reduced prices so rapidly include techniques created by scientists at innovator companies 454, Agencourt, and Solexa. By 2007, all three of these companies had been purchased by larger companies: Roche, Applied Biosystems, and Illumina. Through investment and further improvement, the larger companies made sequencing more widely available.[3]

Figure 1. Sanger Sequencing



Above is a schematic of Sanger sequencing. Each of 4 test tubes containing strand-terminating DNA bases for adenine, cytosine, thymine, and guanine generate strands of DNA that correspond with the position of a base. Gel electrophoresis then orders the strands by length, and the positions of each base pair on the strand of DNA under analysis is revealed.

NGS techniques include[3,6]:

- DNA pyrosequencing, which relies on detection of visible light produced by luciferin when a base integrates into the DNA strand under analysis. Pyrosequencing

became widely available in 2008 with Roche's 454 GS FLX Titanium system. This system was initially able to read 700 million base pairs in 24 hours. By 2009, after several upgrades, the system was able to read 14 billion base pairs in a 24-hour period.

- Sequencing by Oligo Ligation Detection (SOLiD), which relies on fluorescent strands of complementary DNA and an enzyme called ligase to read DNA two bases at a time. SOLiD became widely available in late 2010 with the SOLiD 5500xl sequencing system from Applied Biosystems. A 7-day run of the SOLiD sequencer reads 120 billion base pairs.
- Sequencing by synthesis (SBS), which relies on a method of replicating DNA while it is bound to a 2-dimensional surface (bridge amplification), and reading the surface-bound DNA sequence with an electronic photosensor as fluorescent bases pair with the captive DNA. SBS became widely available in early 2010 with the Illumina's HiSeq 2000 sequencing system. A 3- to 10-day run of the HiSeq 2000 sequencer reads 600 billion base pairs.

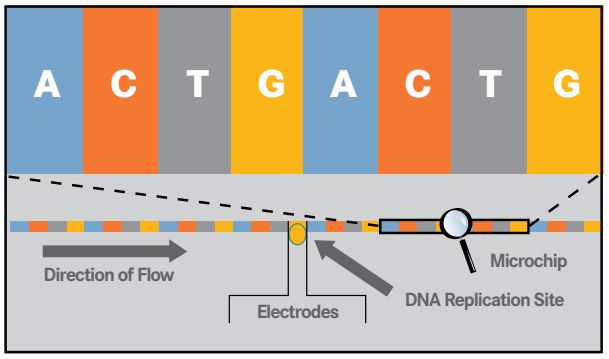
Even these techniques are becoming outmoded with the newest incarnation of sequencing technology: semiconductor sequencing. These systems work by bathing replicating DNA in one new base at a time. The system then electronically measures a subtle drop in pH when a new base binds to the growing DNA strand (**Figure 2**). Semiconductor sequencing systems are smaller and less expensive than earlier NGS systems, making them available to smaller laboratories and even clinics, but they are also less powerful than the larger, more expensive systems.[3]

The first semiconductor system, Ion Torrent's Ion Personal Genome Machine, became available commercially in 2010, and was soon followed by Illumina's 2011 launch of the MiSeq device. Such systems were important in characterizing and containing a strain of pathogenic, antibiotic-resistant *Escherichia coli* that spread through Germany in the summer 2011.[3,6,7] These smaller systems are also helpful in detecting mutations associated with cancers to predict disease course and individualize treatment.[3]

Of the two existing semiconductor sequencing systems, only the MiSeq device has obtained FDA approval for genetic testing, specifically for the *CFTR* gene in patients with suspected cystic fibrosis. BRCA testing is not yet approved by the FDA using semiconductor sequencing systems, although it is technically possible.[8]

For instance, in a trial of the Ion Torrent Personal Genome Machine for the detection of *BRCA1* and *BRCA2* mutations in patients with breast cancer, investigators identified *BRCA1* and *BRCA2* genes with 98.6% sensitivity, 95% confidence, and 96.9% specificity.[9] In this context, NGS is important because it could lead to rapid identification of high-risk mutations, including *BRCA1* or

Figure 2. Semiconductor Sequencing



BRCA2. Knowing that these high-risk mutations are present, more aggressive treatment could be pursued, with radical mastectomy instead of breast-conserving surgery, for instance.[10]

One major advantage of NGS technology is that it enables testing, not just for a single susceptibility gene, but for a group of susceptibility genes simultaneously. In 2158 individuals tested for *BRCA1/2* genes, investigators tested for the presence or absence of 25 genes suspected to be associated with inherited cancer including *BRCA1*, *BRCA2*, *CHEK2*, *ATM*, and *PALB2*. By testing patients for a larger group of genetic cancer susceptibility genes, investigators identified mutations of genes other than *BRCA1/2* in 4.3% of cancers, the majority of which (3.9%) were detected in patients with breast and ovarian cancer.[11]

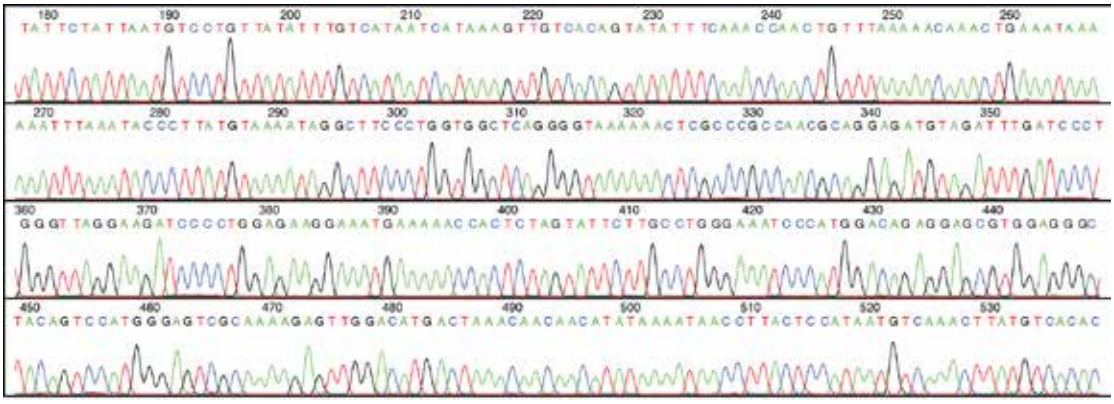
The availability of these multigene panels in patients with breast cancer is made possible, in part, by a Supreme Court decision that invalidated the ability of companies to patent DNA sequences found in nature.[12,13]

Prior to this ruling, Myriad Genetics had been the only source of BRCA gene testing in the United States for a decade. The reason for Myriad's monopoly on the BRCA testing market involved a series of legal battles that began in 1997, when a company that had patented the BRCA1 consensus sequence, OncorMed, sued Myriad for developing a BRCA test using OncorMed's patented sequence. The companies later settled out of court.[12,13]

Myriad later filed suit against the University of Pennsylvania, where researchers had also developed a BRCA test. As a result of these legal skirmishes, nine laboratories stopped offering BRCA testing in the United States, and Myriad gained a de facto monopoly. By 2013, Myriad had generated over $2.8 billion in revenue through BRCA testing and had tested over 1 million women for the mutation.[12,13]

By May 2009, The American Civil Liberties Union and the Public Patent Foundation sued Myriad, and the suit reached the Supreme Court in a session held from 2012 to 2013. After much debate, the Supreme Court ruled that

Figure. DNA Sequence



The technology of DNA sequencing was made faster and less expensive as a part of the Human Genome Project.

DNA segments are not patentable merely because they have been isolated. However, the Supreme Court did decide that complementary DNA, which is generated artificially, is patentable because it does not occur naturally. As a result of the decision, many laboratories across the United States began offering BRCA testing. The ruling also made it possible for companies to explore multigene panels that go beyond testing for a single gene.[12,13]

Despite the potential for multigene testing and NGS techniques to reduce the cost of BRCA testing, Myriad remains competitive by differentiating its BRACAnalysis CDx test as the only companion diagnostic test approved for use with Lynparza (olaparib), a poly ADP-ribose polymerase (PARP) inhibitor for heavily pretreated patients with ovarian cancer who test positive for the *BRCA* mutation. Use of other techniques or other tests to determine whether or not BRCA is present could lead to noncoverage of the drug.[14]

Although Myriad is still an important provider of genetic testing for *BRCA*, use of the BRCA test in connection with a drug does not prevent other companies to make further advancements in testing for *BRCA* and associated genes, including advances in multigene assays with NGS techniques. Without invalidation of the Myriad patent, testing for multiple genes to evaluate cancer risk in breast and ovarian cancer and the advancement of NGS science in cancer care would be severely limited.

REFERENCES

1. Sanger F, Coulson AR. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *J Mol Biol.* 1975;94(3):441-448.
2. Cold Spring Harbor Laboratory. Early DNA Sequencing. http://www.dnalc.org/resources/animations/sangerseq.html. Accessed January 27 2015.
3. Liu L, Li Y, Li S, et al. Comparison of next-generation sequencing systems. *J Biomed Biotechnol.* 2012:251364.
4. Trujillano D, Weiss MER, Schneider J, et al. Next-generation sequencing of the BRCA1 and BRCA2 genes for the genetic diagnostics of hereditary breast and/or ovarian cancer [published online December 31, 2014]. *J Mol Diagn*. DOI: http://dx.doi.org/10.1016/j.jmoldx.2014.11.004.
5. Wetterstrand KA. DNA sequencing costs. National Human Genome Research Institute. http://www.genome.gov/sequencingcosts/. Accessed January 27, 2015.
6. Mardis ER. Next-generation DNA sequencing methods. *Annu Rev Genomics Hum Genet.* 2008;9:387-402.
7. Rohde H, Qin J, Cui Y, et al. Open-source genomic analysis of Shiga-toxin-producing E. coli O104:H4. *N Engl J Med.* 2011;365(8):718-724.
8. FDA allows marketing of four "next generation" gene sequencing devices. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm375742.htm. November 19, 2013. Accessed January 27, 2015.
9. Costa JL, Sousa S, Justino A, et al. Nonoptical massive parallel DNA sequencing of BRCA1 and BRCA2 genes in a diagnostic setting. *Hum Mutat.* 2013;34(4):629-635.
10. Pilgrim SM, Pain SJ, Tischkowitz MD. Opportunities and challenges of next-generation DNA sequencing for breast units. *Br J Surg.* 2014;101(8):889-898.
11. Tung N, Battelli C, Allen B, et al. Frequency of mutations in individuals with breast cancer referred for BRCA1 and BRCA2 testing using next-generation sequencing with a 25-gene panel. *Cancer.*2015;121(1):25-33.
12. Cook-Deegan R, Niehaus A. After Myriad: genetic testing in the wake of recent Supreme Court decisions about gene patents. *Curr Genet Med Rep.* 2014;2(4):223-241.
13. *Association for Molecular Pathology et al. v Myriad Genetics, Inc., et al.* 569 US 12–398 (2013). http://www.supremecourt.gov/opinions/12pdf/12-398_1b7d.pdf. Published October 2012. Accessed January 27, 2015.
14. FDA approves Lynparza to treat advanced ovarian cancer. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm427554.htm. December 19, 2014. Accessed January 27, 2015.

Company Profile: *Foundation Medicine*

Foundation Medicine is a Cambridge, Massachusetts, company that is attempting to use the power of next-generation sequencing (NGS) to build a business in genomic profiling for targeted medicine. Founded in 2009, Foundation has two broad-based tests on the market for finding gene mutations that could be triggers for solid and blood tumors. The company obtains biopsies, blood, and bone marrow samples from patients with cancer, runs the tests to see which gene mutations commonly associated with cancer may be responsible, and provides a report identifying current therapies and therapies in development that may have value.

Foundation Medicine employs ultra-fast NGS to perform its gene testing, reaping the advantages of higher processing power, and claims its assays are accurate and comprehensive. Foundation's tests scan for multiple gene mutations simultaneously, fueling the company's marketing claim that it saves patients money and time over rival tests that check for far fewer mutations.

Foundation Medicine's original test, brought to market in June 2012, is called FoundationOne, and it examines the DNA coding sequence of 315 genes plus introns, or DNA segments, from 28 other genes that are known to be associated with solid cancer development.

In December 2013, the company brought its hematologic cancer test FoundationOne Heme to market, which detects genomic alterations and other mutations in more than 400 cancer-related genes, while also checking for potential gene fusions. The test is billed as an aid in identifying therapy for leukemia, lymphoma, and myeloma, and also sarcomas and pediatric cancers.

In mid-January 2015, Roche announced plans to acquire a majority stake in Foundation Medicine for $1.03 billion, through the acquisition of 15.6 million shares for $780 million and an additional 5 million in newly issued shares for $250 million. Additionally, under the newly formed partnership, Roche will sponsor broad research and development activities conducted by Foundation Medicine, with the potential of more than $150 million in research funding. The deal is expected to close in the second quarter of 2015.

Targeted therapy such as NGS has pushed out the boundaries in the fight against cancer, as it is thought to provide doctors better odds in choosing treatments that will work for their patients. Many cancer medications work in only a portion of patients who receive them. Gene-based therapy is thought to give doctors better insight into which medicines will work with certain patients, and it is also considered valuable in providing clues as to potential treatments that doctors might not have thought to try without the knowledge from the DNA test.

For example, Foundation Medicine has cited the case of a young woman with Li-Fraumeni syndrome and advanced lung cancer that was refractory to many types of conventional chemotherapy. Before a DNA test was conducted doctors were unable to determine what might work. Following a sequencing test of over 200 genes and introns, doctors found that the woman's tumor harbored certain gene mutations with known links to cancer. They then prescribed an inhibitor, afatinib, which led to what researchers described as a "rapid, complete and durable" recovery.[1]

Proponents of DNA tests say they also help doctors understand what medicines may not be effective for certain patients.

At the 2014 ASCO annual meeting in Chicago, Illinois, this year, Foundation Medicine presented the results of a study showing that doctors armed with FoundationOne DNA sequencing results for 132 cancer patients decided

to change therapy for 36 of them. The patients all suffered from refractory metastatic advanced solid tumors of various types, including cancer of the breast, lung, and colon.[2]

Gene profiling is thought to contribute to an understanding of potentially new off-label uses for drugs, and it can also help doctors match patients with drugs that are as yet unapproved but undergoing medical trials. Increasingly, the FDA is approving the use of certain drugs only with an accompanying DNA sequencing test to verify that the drug in question may have efficacy for the patient involved.

However, concerned about the growing role of laboratory-based testing such as Foundation's, and to ensure that the medical guidance patients receive from these tests is as reliable as possible, the FDA in July 2014 announced plans to establish an oversight framework and guidelines that would encompass genetic testing. "Today these tests may compete with FDA-approved tests without clinical studies to support their use," the FDA said.[3]

If usage by doctors is a form of endorsement, FoundationOne and FoundationOne Heme have the support of many within the medical community. The company reported in its most recent financial filing that 2100 physicians from both large academic centers and community-based practices have ordered FoundationOne and FoundationOne Heme for their patients over the past 2 years.[4]

Orders are growing, too. For the 9 months ending in September 2014, the company provided results of 20,600 individual tests ordered by physicians and biopharmaceutical customers, a robust increase from 7500 tests for the comparable 9-month period ending September 2013.[4]

Although use of the tests is rising rapidly, one key to the company's viability as a business enterprise will be whether it can make money. Foundation is a commercial-stage company, and although it has a list price for its tests of $5800 for FoundationOne and $7200 for FoundationOne Heme,[5] the company is actually earning substantially less for each test it performs, and in many cases the revenue has yet to materialize, owing to the newness of genomic testing and the lack of established reimbursement pathways for such medicine.

Many commercial payers do not have established policy for Foundation's broad-based test, and many reimbursement decisions are based on the amounts paid for other types of diagnostic testing or are negotiated directly. "Coverage and payment is determined by each third-party payer on a case-by-case basis," the company reported in its most recent financial statement.[4]

In the same statement, the company reported that the average revenue for the FoundationOne and FoundationOne Heme tests combined was just $3600 over the 9-month reporting period. However, that figure was based on tests for which revenue was received. There were many more tests for which revenue was still outstanding.

For example, there were 1685 tests for patients covered by Medicare for which payment was not received because Medicare has not allowed reimbursement for these tests. In fact, Foundation Medicine has been applying for reimbursement from Medicare since November 2013 and has an outstanding appeal for a coverage decision.

The company said that overall it has not received payment for roughly 15,000 tests (in total, since inception, not for the 9-month period). However, in October 2014, the company announced that nonprofit insurer Priority Health had agreed to cover both of Foundation's tests for blood and solid tumor cancers. This made Priority Health the first health plan in the country to cover FoundationOne and FoundationOne Heme. Foundation Medicine stated at the time that it viewed this decision as a validation of the clinical utility

Genes Examined

The FoundationOne test examines multiple genes, with 17 main alterations that are frequent red flags in the cancer spectrum.

Gene	Associated Malignancy
AKT1	Breast, ovarian, and colorectal cancers
APC	Turcot syndrome, which signals a higher risk for colon cancer.
ATM	Breast cancer, promotes tumor development.
BRCA1	Fallopian tube cancer, male breast cancer, and pancreatic cancer
BRCA2	Ovarian cancer, prostate cancer, pancreatic cancer, fallopian tube cancer, male breast cancer, and melanoma.
CTNNB1	Desmoid tumors, philomatricomas (skin tumors), and colorectal, liver, thyroid, ovarian, endometrial and brain cancers.
HER2	Approximately 25% of breast cancers
EGFR	Lung cancer and to a lesser extent, ovarian, gastric and head and neck cancers.
HRAS	Costello syndrome, which leads to cancerous and noncancerous tumors
KDR	Infantile capillary hemangiomas, a form of tumor.
KIT	Leukemia and lymphoma and also gastrointestinal tumors
KRAS	Pancreatic, lung, and colorectal cancers.
MET	Renal carcinomas, gastric cancer and a susceptibility to autism.
PIK3CA	Ovary, breast, lung, brain, and stomach cancers
PTEN	Cowden syndrome, which involves higher risk of breast cancer, thyroid cancer, and cancer of the uterine lining.
TP53	Breast cancer, and Li-Fraumeni syndrome
CHEK2	Prostate, lung, colon, kidney, thyroid, and ovarian cancers and also in some brain tumors and in a type of bone cancer.

of using genomic profiling to inform treatment plans.[6]

In July 2014, the company announced that the New York State Department of Health had approved its solid tumor and hematologic malignancy tests, which had previously been accepted only conditionally in the state, where an independent regulatory review is required for laboratory tests.[7]

Recently, Foundation Medicine participated in a 2-year validation study of the company's approach to targeted medicine. The study, published in the journal *Nature Biotechnology* in October 2013, examined 2221 patient samples of breast, lung, colorectal, head and neck, prostate, and other cancers, and tested 189 genes for "smoking gun" mutations. The researchers found clinically actionable mutations in 76% of the samples, according to the study, leading them to conclude that the FoundationOne test can be "as accurate as clinical assays currently in use," while also testing for a much larger slate of "clinically relevant cancer genes."[8,9]

Foundation Medicine has expressed confidence that its broad-based gene testing platforms represent a niche in a marketplace crowded with competitors offering DNA sequencing tests that examine smaller sets of genes and mutations. The company has managed to attract investment capital from prominent businessmen, including Microsoft founder Bill Gates and Russian entrepreneur and venture capitalist Yuri Milner.

REFERENCES

1. Jia Y, Ali SM, Saad S, et al. Successful treatment of a patient with Li-Fraumeni syndrome and metastatic lung adenocarcinoma harboring synchronous EGFR L858R and ERBB2 extracellular domain S310F mutations with the pan-HER inhibitor afatinib [published online May 16, 2014]. *Cancer Biol Ther.* 2014;15(8):970-974. doi: 10.4161/cbt.29173.
2. Foundation Medicine announces initial data demonstrating clinical utility of FoundationOne® in advanced solid tumors; clinical data to be presented at 2014 ASCO annual meeting. Cambridge, MA: Foundation Medicine; May 31, 2014. http://investors.foundationmedicine.com/releasedetail.cfm?releaseid=851588. Accessed January 26, 2015.
3. FDA takes steps to help ensure the reliability of certain diagnostic tests. July 31, 2014. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm407321.htm. Accessed January 26, 2015.
4. Foundation Medicine, Inc. 10Q quarterly filing. September 2014. http://yahoo.brand.edgar-online.com/EFX_dll/EDGARpro.dll?FetchFilingHtmlSection1?SectionID=10302782-814-149795&SessionID=wHmS6q-zAf89Wk7. Accessed January 26, 2015.
5. FoundationOne. How is the genomic profiling approach different? http://foundationone.com/learn.php. Accessed January 26, 2015.
6 Priority health is first health plan to cover FoundationOne® and FoundationOne® Heme to enhance precision cancer treatment. Cambridge, MA: Foundation Medicine; October 16, 2014. http://investors.foundationmedicine.com/releasedetail.cfm?releaseid=876650. Accessed January 26, 2015.
7. Foundation medicine receives approvals from New York State Department of Health for FoundationOne® and FoundationOne Heme®. Cambridge, MA: Foundation Medicine; July 17, 2014. http://investors.foundationmedicine.com/releasedetail.cfm?releaseid=860363. Accessed January 26, 2015.
8. Foundation medicine announces publication of analytic validation study of FoundationOne™ in Nature Biotechnology. Cambridge, MA: Foundation Medicine; October 20, 2013. http://investors.foundationmedicine.com/releasedetail.cfm?releaseid=798492. Accessed January 26, 2015.
9. Frampton GM, Fichtenholtz A, Otto GA, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing [published online October 20, 2013]. *Nature Biotechnology.* 2013;31:1023-1031. doi:10.1038/nbt.2696.

Illumina

Illumina Inc, sells the majority of the world's genome-sequencing machines and a wide array of products and services designed to make use of genetic information. It is estimated that machines from the San Diego, California-based company have sequenced 90% of all of the genomes that have ever been decoded, and that percentage may only increase. Early this year–less than a decade after the completion of the 13-year, $3 billion effort to sequence the first human genome–Illumina unveiled the HighSeq X, a device that can sequence an entire genome in about a day and, more important, at a cost of just $1000.

The company's primary customers to date have been researchers, but many believe that the $1000 price point will make genetic sequencing a common technology for clinical medicine, and CEO Jay Flatley says that his company will "blow open the cancer market" in the next couple of years. Illumina already offers researchers a number of cancer "panels" that check tumor samples for important genetic variations.

Researchers who want a quick way to check samples for a fairly small number of variations that are most important to cancer treatment, for example, can use the TruSeq Amplicon Cancer Panel, which needs only small tissue samples to detect mutations to 48 cancer genes such as *BRAF*, *KRAS*, and *EGFR*.

Researchers who are looking for low-frequency variants in solid tumor types, meanwhile, can use the

Illumina's Current Products

Product	Description
TruSight Tumor Panel	Identifies low-frequency variations across 26 genes in a variety of solid tumor types that includes lung, colon, and ovarian cancer as well as melanoma. The panel provides coverage of complete exons selected from CAP and NCCN guidelines, and Illumina says it offers a limit of detection below 5% variant allele frequency across 174 amplicons.
TruSeq Amplicon Cancer Panel	Looks specifically for a relatively small number of highly important somatic mutations, mutations to cancer-related genes such as *BRAF, KRAS* and *EGFR*. The panel detects 48 mutations from a single small genetic sample. As with the TruSight Tumor panel, it is sensitive to infrequent mutations and it works with FFPE samples that have suffered significant genetic damage.
TruSight Myeloid Sequencing Panel	Designed by a consortium of blood cancer experts to identify genetic mutations that are relevant to acute myeloid leukemia, myelodysplastic syndrome, myeloproliferative neoplasms, chronic myelogenous leukemia, chronic myelomonocytic leukemia, and juvenile myelomonocytic leukemia. The panel covers 15 full genes (exons only) and the cancer "hotspots" of 39 additional genes.
TruSight Cancer Panel	Unlike any of the other tests, it starts with healthy tissue and provides information about 94 genes and 284 single nucleotide polymorphisms that appear to increase a patient's predisposition to both common and rare types of cancer. Illumina worked with The Institute of Cancer Research in London, UK, to analyze existing research and decide which genetic variations were validated well enough to include in the panel.
TruSeq Custom Amplicon	Allows researchers to gather genetic information about hundreds of genomic regions covering up to 650 kb of cumulative sequence. The panel works with a wide range of sample types, including FFPE samples. This fully integrated DNA-to-data solution includes probe design and ordering using DesignStudio, an easy-to-use online software tool that provides dynamic feedback to optimize design and region coverage.

CAP, College of American Pathologists; FFPE, formalin-fixed paraffin-embedded; NCCN, National Comprehensive Cancer Network.

TruSight Tumor Panel, while those who are interested in genetic mutations in blood cancers can try the TruSight Myeloid Sequencing Panel.

Illumina also has a product for researchers who are investigating connections between the genetics of healthy tissue and the risk of cancer. The TruSight Cancer Panel sequences all of the genes that are currently known to affect a patient's predisposition to cancer.

Illumina has designed all of these tests to work on its sequencing machines, which range from industrial workhorses to single-lab tools.

At the top of whole-genome range are the HighSeq X machines, which cost $1 million apiece and must be bought in 10-unit groups that can sequence 20,000 full genomes per year. NexSeq 500 machines, on the other hand, are small enough to sit on a desk and cost $250,000.

The MiSeq device is smaller still and targeted more to sequencing small bits of genetic material than assembling entire genomes. It can perform all of the cancer panels listed above, though not as quickly as larger machines. Still, they may prove fast enough for some small practices, or even some commercial testing centers, and such operations may soon be able to use Illumina's machines.

Late last year, an Illumina product called the MiSeqDx became the first next-generation sequencing (NGS) device to win approval from the US Food and Drug Administration (FDA) for use in clinical diagnostics. That single approval does not immediately enable commercial test providers to perform existing research assays with the approved device, however. In many cases, the FDA must approve both the specific clinical assay and the machine that performs it.

Technically, any company can design an assay that works with the new sequencing technology, but Illumina has an obvious head start. Its assays already work on the approved machine, though the company

has yet to have any of them specifically cleared by the FDA for commercial use rather than research projects.

That said, the company announced plans to ask the FDA to approve other genome-sequencing machines for use in clinical test labs, larger machines that can sequence far more material far more quickly than the MiSeqDx. Indeed, Flatley told investors late last year that Illumina had made a "strategic decision" to ask for FDA clearance of the company's HiSeq 2500 device, which produces more than 60 times the data output of its smaller counterpart.

Illumina executives have also said that they view cancer—along with infectious diseases and organ transplantation (where donors and recipients must be genetically similar)—as one of the three biggest markets for clinical assays that use NGS technologies.

Existing testing methods work well enough for extracting a few pieces of genetic information from tissue samples, but oncologists often want far more than a few pieces of information about individual tumors, and their demands will continue to grow.

Researchers have already discovered 54 genetic variants that promote the development of cancer and 71 that suppress it through 12 different signaling pathways. Ongoing research appears certain to increase those figures dramatically.

Of course, only a relatively small number of existing medications target any of those genetic variations, but there are hundreds of experimental drugs being developed around the world that do target particular mutations. There are also many drugs that were not specifically designed to target any particular genetic variant that work dramatically better on some tumors than others. Broader, better genetic tests could identify the reason for such discrepancies and help doctors prescribe such medications to patients who would actually benefit from them.

Illumina has already begun making a play for one particular area of the tumor testing market: the creation of genetic tests that are used to predict patient response to targeted therapies. Many newer therapies are approved in conjunction with test kits designed to predict whether patients will respond to them, and Illumina has signed a deal with Amgen to develop a NGS companion diagnostic for Vectibix (panitumumab), an antibody therapy for colorectal cancer that works only on tumors with certain genetic profiles.

The FDA initially approved the medication in 2008 as a second-line monotherapy for patients with *EGFR*-expressing tumors. However, further analysis of drug trials led the FDA to narrow its indication to patients without a *KRAS* gene mutation. Subsequent trials on well-targeted populations—patients with wild-type

Courtesy of illumna Inc.

KRAS cancer—then convinced regulators to approve panitumumab as a first-line treatment, along with FOLFOX chemotherapy, for metastatic colorectal cancer.

few months after beginning its partnership with Amgen, Illumina announced that it would work with AstraZeneca, Janssen Biotech, and Sanofi to develop a "universal oncology test system." The resulting assay, which will simultaneously measure multiple genetic variants, will be used to support the clinical trials of all three companies. Better tests will help those companies determine exactly which patients will benefit from which treatment and, thus, secure regulatory approval faster and help more patients. Drugs developed with such an assay could well be approved along with the universal assay itself or, perhaps, a simplified assay variant for each particular medication.

A number thought leaders among cancer researchers and patient advocates would very much like to replace such individual tests for individual medications with broader assays that would be able to determine the treatment most likely to work for any particular patient with any particular tumor type.

Many obstacles could hinder the acceptance of such a test, but Illumina officials say they are taking steps toward making it a reality, not only by developing the universal assay for its partners in the pharmaceutical industry, but also by working with some of those thought leaders to set standards and define regulatory frameworks for panel-based assays that use NGS technology. Of course, Illumina's efforts to develop new cancer assays and sell gene-sequencing machines to other makers of oncology assays are just one piece of a large pie.

Illumina owns Verinata Health, which provides prenatal tests that offer information about a wide variety of conditions without the risks of amniocentesis, which

requires doctors to stick a needle into the expectant mother's uterus. Analysts believe that such tests could eventually become a multibillion-dollar market.

Illumina just won a contract from the United Kingdom to help with a massive research project that will sequence 100,000 full genomes, mine the results for information about every sort of genetic malady, and, in a few years, begin making genetic testing a regular component of British healthcare services.

Illumina works with doctors in the United States to provide a full-genomic sequencing and analysis for all patients who want to know what 1600 of their individual genes say about their risk of contracting 1200 different conditions. The number of genes and conditions, naturally, continue to rise while the price continues to fall. Observers predict that when the commercial price falls below $1000, it will become very popular.

It is, in other words, difficult to find an area of medicine that is completely untouched by Illumina's products and services, or an area of medicine that won't become more reliant on the company as it becomes more reliant on genetic information. Even now, oncology uses such data far more than other specialties, and although Illumina's impact on the specialty has largely been confined to research projects so far, clinical practitioners will soon have access to products and services from the company.

Indeed, given Illumina's dominance in the market for NGS machines, most oncologists will move from simple genetic assays that provide only a handful of data points to broad panels that provide pages of data, and will almost certainly be using the company's products, whether they know it or not.

Caris Life Sciences

Many companies in the highly competitive life sciences field find it necessary to offer the latest technology while simultaneously investigating new methods of disease detection. Caris Life Sciences features its Molecular Intelligence platform while also developing new technologies to identify tumors early through what it calls its Carisome Microvesicle Technology.

For many years, oncologists have relied on three primary methods of identifying cancerous tumors: surgery, imaging, and examining tissue samples collected via biopsy. In recent years, however, oncologists have begun collecting microvesicles circulating in patients' blood and examining the molecular profile of these microvesicles to identify tumors early.

In the 1970s, researchers showed that microvesicles contained evidence of Hodgkin disease in a patient. Since then, researchers have reported that tumor-derived microvesicles (TMVs) have a significant effect on tumor growth, survival, and the spread of disease.

The potential to use TMVs for the diagnosis and treatment of certain cancers could be significant. As TMVs are released into blood and urine they have the potential to serve as circulating biomarkers of disease progression.

Because the genomic and proteomic profiles of tumors change as cancer progresses and in response to treatment, TMVs could be useful to oncologists staging a patient's disease and when assessing the patient's response to therapy.

Tumors shed a substantial amount of microvesicles—so many in fact that a milliliter of blood might contain between a million and several billion. These TMVs are larger than proteins but smaller than circulating cancer cells, a factor that led researchers to discard them as debris.

Recognizing the potential importance of TMVs for the detection and treatment of cancer, Caris Life Sciences in Irving, Texas, has developed a patent-pending testing platform called Carisome Microvesicle Technology to identify and characterize disease-associated circulating microvesicles (which it calls cMVs) in the blood. This clinical platform can be used for early diagnostic testing, disease monitoring, and to evaluate cancer patients' response to therapy, the company said.

In 2011, researchers Adam S. Kibel, MD, and Gerald L. Andriole, MD, from the division of Urology, Department of Surgery at Washington University in St. Louis, Missouri, and colleagues presented research on the value of using cMVs to detect prostate cancer in at-risk patients. The purpose of the research was to determine whether a cMV-based immunoassay could be used to identify those patients who have prostate carcinoma. This test could be important to urologists because of the limitations inherent in the commonly used prostate-specific antigen CARIS (PSA) test.

Using the PSA test for prostate cancer screening often results in detection of nonmalignant processes, such as infections and benign prostatic hyperplasia. In addition, the PSA test can show high PSA levels, a potential cause for concern that leads to more testing.

For the study, Kibel, Andriole, and colleagues used an assay that enables them to isolate, capture, and characterize prostate cancer–specific cMVs. The assay they developed selects antibodies to protein biomarkers based on their ability to differentiate between men with and men without prostate cancer.

The researchers described cMVs as being heterogeneous membrane-bound structures approximately 30 nm to 1000 nm in diameter. A variety of cell types in different tissues secrete cMVs into the blood and other bodily fluids. The microvesicles play a role in intercellular communication, inflammation, immune response modulation, and coagulation. They also have been implicated in tumor progression, invasiveness,

Caris Molecular Intelligence Technologies Offered

IHC testing	To determine the level of protein expression
Gene copy number analysis	CISH[a]
	FISH[a]
	qPCR to amplify and quantify DNA molecules
Methylation analysis	RNA fragment analysis
NGS	Microsatellite instability
Turn-around time (from case activation)	7-14 days
Microdissection performed on all cases	Yes; results in ~25% increase in tumor nuclei
Sample requirements for full profile	100 ·m to 220 ·m tissue, ≥20% tumor after microdissection, 25-55 unstained slides or in a FFPE block of tissue
Sample requirements for NGS	≥40 ·m tissue, ≥20% tumor content, on 8-10 unstained slides or in FFPE block
Attributes of NGS	
Sensitivity (base substitutions)	>99% (≥10% mutant allele frequency)
Sensitivity (insertions-deletions)	>99% (≥10% mutant allele frequency)
Sensitivity (copy number variations)	Tested by FISH/CISH; meets ASCO/CAP clinical threshold (meaning 6 copies per cell and ratio determination)
Specificity	>99%
Positive predictive value	>99%
Negative predictive value	>99%
Detects mutations	Yes
Confirms wild type	Yes
Average depth of coverage	>1500X
Amount of DNA	20 ng-200 ng
Number of copies of tumor DNA (20% tumor nuclei)	23,200 (enriched to 45% tumor with microdissection)
Probability of missing a mutant with 25% mutant frequency in samples with 25% tumor density	0.10%

ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; CISH, chromogenic in situ hybridization; FFPE, formalin-fixed paraffin-embedded; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NGS, next-generation sequencing; qPCR, quantitative polymerase chain reaction.

aFISH and CISH detect gene deletions, amplifications, translocations, and fusions.
The Caris laboratory is CLIA- and CAP-certified and certified to ISO 15189.

Source: Caris Life Sciences, Irving, TX; 2014.

and angiogenesis, the researchers reported.

Perhaps most important, the researchers said that cMVs contain a variety of molecules that reflect their cellular origin. Therefore, cMVs released by tumor cells may provide a source of potential tumor-associated biomarkers, they wrote.

For the study, the researchers evaluated the sensitivity and specificity of their assay on frozen plasma samples from men with biopsy-confirmed, nonmetastatic prostate cancer (n = 331) and controls (n = 197). Prostate-specific cMVs were captured, analyzed, and blindly classified as positive, negative, or borderline. The assay was successfully run on 528 of 649 (84%) cases and controls.

The researchers demonstrated that the cMV assay has high specificity and sensitivity for detecting prostate cancer and outperforms PSA. They recommend further validation in a larger prospective cohort, and note that this assay could significantly improve prostate cancer detection, thus enabling physicians to make more informed decisions regarding invasive testing and therapies.

Molecular Intelligence

Caris also has developed a tumor-profiling service called Caris Molecular Intelligence, which uses a multitechnology approach to provide oncologists with treatment options for personalizing cancer care. The technologies include next-generation or Sanger sequencing, protein analysis through immunohistochemistry (IHC) testing, and gene copy number analysis using chromogenic in situ hybridization (CISH) and fluorescence in situ hybridization (FISH) and quantitative polymerase chain reaction (qPCR). Immunohistochemistry testing determines the level of protein expression. Fluorescence in situ hybridization and CISH detect gene deletions, amplifications, translocations, and fusions. Next-generation sequencing is used to determine the DNA sequence of a specimen and is designed to detect mutations. And qPCR amplifies and quantifies DNA molecules.

In October 2014, researchers presented two studies at the 2014 Chicago Multidisciplinary Symposium in Thoracic Oncology that showed that Caris Molecular Intelligence tumor profiling service was used successfully in analyzing lung tumors. The findings are potentially significant for managing the care of patients with small-cell lung cancer (SCLC), non-small-cell lung cancer (NSCLC), and neuroendocrine tumors (NETs), according to Caris.

Multiplatform molecular profiling is important because the Clinical Practice Guidelines in Oncology from the National Comprehensive Cancer Network call for multiplex testing to replace testing for epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*).

In one study, researchers used the Molecular Intelligence services to identify genomic alterations and mutations of multiple genes linked to SCLC and lung NETs, the company said. In the other study, researchers assessed aberrations of NSCLC biomarkers that may account for drug resistance and may help oncologists identify strategies for combination therapies that may be effective in some patients. The NSCLC study comprised 6785 cases and is one of the largest analyses in the United States of biomarkers for this disease.

The SCLC/NET study is important because these tumors are uncommon, meaning that oncologists have limited experience with them, said the lead author of the SCLC/NET study, Stephen V. Liu, MD. By demonstrating how molecular profiling can be used to manage the care of patients with lung NETs and SCLC, the study findings suggest that comprehensive molecular analysis should be used for more patients with lung tumors, added Liu, an assistant professor of Medicine in the Department of Hematology/ Oncology, Lombardi Comprehensive Cancer Center, Medstar Georgetown University, Washington DC.

The findings have potentially important therapeutic implications for management of patients with SCLC, NSCLC, and NETs of the lung, as well as for identifying potential mechanisms of drug resistance.