

Molecular & Genetic Testing in Anatomic Pathology

By Sandeep Mukherjee, Ph.D.
Director, Molecular Diagnostics
CellNetix Pathology & Laboratories



For more than 100 years pathologists have been using microscopes to look at abnormal tissue and cells to better diagnose disease. Today, we are evolving beyond cellular examination to identify the root cause of these abnormalities within the genetic material of the cell itself. The mapping of the human genome has driven growth in molecular testing, which now allows laboratories to analyze tumor tissue and other specimens to determine likelihood of recurrence, source of disease

and the best types of therapies to be used. Increased attention is being paid to “personalized medicine” which targets therapies to individual genetic signatures or profiles. These “targeted therapies” represent the most promising advance in cancer treatment yet. There has been a 30% increase in molecular testing in laboratories across the country in the last few years especially in women's health, infectious diseases, organ transplant testing and oncology and this growth is projected to continue.

What is Molecular & Genetic Testing?

Molecular genetic testing spans the entire spectrum from the characterization of cell biology, protein expression and chromosomal rearrangements to the resolution of single abnormalities in the DNA template. Infectious agents can be identified by virtue of unique DNA/RNA sequences. Molecular testing is used not only in diagnosis, but also in monitoring for the effectiveness of therapy and detection of residual disease in various malignancies. Molecular techniques can also predict the effectiveness of some important medications as

well as identify specific targets in individual patients' tumors for new therapeutic modalities (“personalized medicine”).

Molecular testing utilizes sensitive tools that often confirm ambiguous diagnoses suspected by microscopic evaluation, guide therapeutic decisions and assess. For example, the advent of new treatments for certain breast cancers depends on identification of a gene that is amplified and over-expressed in those cancers; the specific gene that is amplified can only be identified by molecular testing.

Molecular Techniques

Molecular testing has impacted clinical practice quite dramatically. Genetic tests are now available for more than 1,700 diseases, up from 1,250 in 2005.¹ Interpretation of slides under the microscope remains the basis of anatomic pathology. However, an expanding menu of molecular tests now complements traditional pathology methods.

Molecular tests are rapid, sensitive and specific and can be performed on almost all specimen types.

Positive Patient Outcomes

When is molecular testing beneficial? The most obvious benefits are in the targeted treatment of diseases such as the cancers described above and the detection of subclinical (difficult to detect) conditions. Molecular tests can indicate disease risk in pre-symptomatic individuals, assess the risk of recurrence, or determine carrier status and the risk for affected offspring. They may also contribute to more precise diagnosis, refine prognostic categories, connect patients to optimal treatment choices, and monitor treatment efficacy.

CellNetix Molecular Program

Cellnetix started our molecular program in late 2010. This has taken considerable investment and we were only able to undertake this as a relatively large, consolidated group (CellNetix is one of the largest physician owned pathology groups in the country). We will briefly review the common molecular tests (and their applications in Anatomic Pathology) that we currently offer at CellNetix, specifically breast, gastric, colon, lung and melanoma targeted tests.

Breast Carcinoma

The HER2/neu gene is amplified in 18-20% of breast carcinomas, which is tied to overexpression of its protein product and malignant transformation. It is an independent marker for poor clinical outcome in newly diagnosed patients; however, breast cancer patients with increased expression of HER2/neu have a good therapeutic response to trastuzumab (Herceptin) (Genentech, San Francisco, California), a targeted antibody-based therapy

for breast cancer and to paclitaxel. These patients also have relative resistance to endocrine therapies such as tamoxifen and other chemotherapeutic agents. The most commonly used molecular test is fluorescence in situ hybridization (FISH) which allows for the examination of genomic DNA in situ.

Gastric/Gastroesophageal Junction Cancer

Amplification of the HER2/neu gene is observed about as frequently in advanced gastric and esophageal adenocarcinoma as in breast cancer. In October 2010, the FDA approved Herceptin in combination with chemotherapy for HER2/neu-positive metastatic cancer of the stomach or gastroesophageal junction, in men and women who have not received prior treatment for their metastatic disease. The current recommendation for people diagnosed with metastatic stomach cancer is to have the HER2 status of their tumors determined.

Colorectal Carcinoma (CRC)

Mutations of the KRAS (Kirsten ras) gene, found in 30% to 40% of CRCs, are important in the development of cancer. Presence of mutations in this gene can predict lack of response to Panitumumab (Vectibix®, Amgen) and Cetuximab (Erbix®, Merck Serono) in patients with metastatic colorectal cancer. This illustrates the need for sensitive and accurate KRAS testing for these patients. At CellNetix, we use a sensitive real-time molecular assay to detect the most common KRAS mutations.

This real time method is also used to detect the other mutation in what

is called the BRAF oncogene. Mutations in BRAF are also observed in colorectal carcinomas; these mutations are responsible for an additional 12-15% of patients who fail to respond to antibody-based targeted therapies. BRAF mutations appear also to be indicative of poor prognosis and testing for mutations in BRAF is recommended under certain circumstances.

Non-Small Cell Lung Cancer (NSCLC)

The Epidermal Growth Factor Receptor (EGFR) gene encodes a cell membrane receptor; it is a target for signals that set off a cascade of downstream effects and the subsequent development of cancer. Identification of EGFR signaling in cancer has led to the development of anti-cancer therapeutics directed against the EGFR protein, including Gefitinib (Iressa®, AstraZeneca) and Erlotinib (Tarceva®, Roche) for non-small cell lung cancer. Current recommendations are that tumors of all NSCLC patients considered for Gefitinib therapy must be tested for the presence of EGFR mutations before the drug can be prescribed, as Gefitinib is unlikely to be effective in patients without EGFR mutations.

The ALK gene encodes another cell receptor protein involved in the development of NSCLC. Through the use of a FDA-approved protocol, the molecular laboratory in CellNetix routinely performs a quantitative test to detect ALK rearrangements (breaks in the gene) via FISH in NSCLC tissue specimens. This is used to aid in identifying those patients eligible for treatment with XALKORI® (Crizotinib, Pfizer), a drug that is specifically inhibits the activity of the ALK gene in lung tumors.

Melanoma

Melanoma is a complex genetic disease, and multiple genetic alterations have been reported to play a role during disease progression. The BRAF gene is mutated in approximately 8% of human tumors, most frequently in melanoma, where the predominant mutation is observed in approximately 50% of melanomas. Like the mutations referred to above, BRAF mutation is one of the primary drivers of malignancy in the tumor. Zelboraf® (Vemurafenib, Genentech) is an orally available, selective BRAF inhibitor approved by the FDA for patients with unresectable or metastatic melanoma that tests positive for the BRAF mutation.

CellNetix Molecular Pathology Services

Compared to other laboratory tests, molecular-based tests are higher complexity, more expensive, and may have additional ramifications for the patient and his or her biological family members. CellNetix's large anatomic pathology laboratory with diverse molecular and surgical pathology capabilities is well positioned to expand the utilization of molecular testing in pathology practice. Our relative size (45 pathologists/250 employees) gives CellNetix the opportunity to be at the forefront of these new testing protocols.

Our Molecular Pathology department started with our women's health subspecialty, and we brought on High-risk HPV testing, testing for the detection of Neisseria gonorrhoeae (GC) and Chlamydia trachomatis (CT), as well as Vaginosis/Vaginitis testing. We have now

also expanded our capabilities in Molecular Oncology. We are up and running with a multitude of FISH and Polymerase Chain Reaction (PCR) -based assays, namely the HER2 FISH for Breast and Gastric Cancer, ALK FISH for Non Small Cell Lung Carcinoma, and Ploidy FISH analysis of Products of Conception. We are also running KRAS mutation assays for Colorectal and Lung Cancer, EGFR mutation for Lung Cancer and BRAF mutation

tests for Metastatic Colorectal cancer and Melanoma.

Conclusion

The impact of molecular diagnostics will be felt throughout the entire healthcare system. As the field of genomics gains broader application, clinicians will need to be educated on the proper utilization and interpretation of molecular tests. The pathologist is expected to be



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a key stakeholder in the future of molecular diagnostics. Pathologists and laboratory professionals need to understand and adopt these new and emerging technologies and their expertise will be critical in this new frontier in medicine. As with many new technologies each day brings more entrepreneurs offering the "latest and best" solutions using molecular technologies to clinicians.

Over time and with appropriately controlled studies some of these assays will in fact show their value in improving patient care or reducing waste. However, our experience shows that more often than not extended study rejects the claims of many new tests as delivering on their marketed promises. Clinicians are encouraged to adopt molecular testing technologies when they have

been properly reviewed and/or recommended by appropriate professional societies.

Reference:

¹National Center for Biotechnology Information. GeneTests. Available at: <http://www.ncbi.nlm.nih.gov/projects/GeneTests/static/whatsnew/labdirgrowth.shtml>.

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