

Molecular and Genetics Testing For Personalized Cancer Treatment

Chuanbo Xu



About the author: Chuanbo Xu, PhD, BVM, PMP, is currently the Director of Project Management at XDx, a molecular diagnostics company in Brisbane, California, where he leads cross-functional team to develop molecular diagnostics for autoimmune disease. Prior to this, Dr. Xu led pharmacogenomics biomarker project for late-stage clinical development of type II diabetes TZD compound at Perlegen Sciences. Prior to that, he held position of up to Senior Director at Genaisance. Dr. Xu has also held a variety of technical and managerial roles at GlaxoSmithKline and Pioneer Hi-Bred int'l.

Dr. Chuanbo Xu received his bachelor degree in Veterinary Medicine from Jilin Agricultural University, Ph.D. in immunology from Chinese Academy of Agricultural Sciences, and a master in Bioinformatics from University of Paris V. He conducted post-doctoral training at Pasteur Institute in France. He is also a certified Project Management Professional by the PMI (Project Management Institute) in Newton, Pennsylvania.

Introduction

Human society has been long dreaming the ability to prevent severe disease outcome from happening by detecting sub-clinical disease earlier. Unfortunately, as in the centuries past, medicine still joins battle with disease only when symptoms arise. Since the discovery of the material and mechanism of genetic information in the 1950's, with the exponential growth of experimental and computing technologies starting in the 1970's, the frontiers of the biological sciences and medicine have been constantly challenged and the speed of data accumulation and knowledge dissemination has been beyond any stretch of imagination. Today, a personalized healthcare approach is taking shape to address each individual's specific disease state and conditions and its impact on the clinical practice is imminent. The current article focuses on the commercially available and medically impacting genomics based diagnostics to achieve personalized healthcare decision-making.

Due to the nature of the high mortality and morbidity of the disease, cancer is one of the pioneering medical fields that employed the genomic tests. There is a spectrum of tests available in this disease area ranging from early screening and diagnosis, to molecular genotyping of the disease states, to disease risk stratification, to treatment efficacy guidance.

Exemplary cancer diagnostic tests

1. HER-2/neu Test for Breast Cancer Treatment

Often cited as the traditional example of personalized medicine applications, Herceptin test was made available since 1998. It is a test that predicts the efficacy of the breast cancer drug, Herceptin (trastuzumab), by detecting the patient population that have the over-expression pattern for the HER-2 (Human Epidermal growth factor Receptor 2) gene. HER-2/neu is an oncogene that is over-expressed in approximately 20% to 30% of breast cancers. Trastuzumab blocks the protein receptors, inhibiting continued replication and tumor growth^[1-3].

The test was first developed using immunohistochemistry (IHC) method^[4]. When more sensitive and accurate method of fluorescence in situ hybridization (FISH) became available, the test was implemented and validated on the new platform^[5]. Currently, the gold standard for assessing HER-2/neu status is still IHC, and the results are scored as 0, 1+, 2+ and 3+ where 0 and 1+ are considered negative results and 2+ and 3+ are considered positive. A positive result suggests that patient is a good candidate for Herceptin therapy. FISH may be used to confirm HER-2/neu positive status by IHC or to help when IHC generates an unclear result.

In addition, the HER-2 test has been adopted for the prescription of other chemotherapies in breast cancer, for example, doxorubicin, cyclophosphamide and paclitaxel^[6]. Patients with a HER-2-positive breast cancer benefited from paclitaxel, regardless of estrogen-receptor status, but paclitaxel did not benefit patients with HER-2-negative, estrogen-receptor-positive cancers.

Recently, the effort using RNAi to simultaneously inactivate thousands of genes in cells that are sensitive to a specific cancer drug to discover the mechanism for resistance to Herceptin is under way, and there are plans to develop a microarray-based diagnostic that can identify resistance-associated biomarkers. Berns et al. present evidence that activation of the PI3 kinase pathway, either via loss of the tumor suppressor PTEN or through oncogenic stimulation of PIK3CA, can mediate trastuzumab resistance^[20]. The pathway is mutated in up to half of all breast cancer tumors.

2. BRCA Gene Tests for Breast and Ovarian Cancer Susceptibility

The discovery of the familial breast cancer susceptibility gene, BRCA1, in the early 1990s^[7-9], and the subsequent commercialization of the sequencing test on the mutations of this gene represent a significant progress in cancer early detection and prevention. This gene encodes a nuclear phosphoprotein that plays a role in maintaining genomic stability and acts as a tumor suppressor. The encoded protein combines with other tumor suppressors, DNA damage sensors, and signal transducers to form a large multi-subunit protein complex known as BASC for BRCA1-associated genome surveillance complex. Researchers have established that mutations in this gene are responsible for approximately 40% of inherited breast cancers and more than 80% of inherited breast and ovarian cancers. Subsequently, another gene, BRCA2, was found to be associated with the risk of developing breast cancer^[10,11]. Although the underlying mechanism and genetics are believed to be very different^[12], the two genes are the most important risk factors for breast cancer in the current knowledge.

Commercial test, called BRACAnalysis, on these two genes is available to indicate a predisposition to hereditary breast and ovarian cancer (HBOC). The test is prescribed to the patient who has family history of the cancer of the organ of breast or ovary. The first order blood relatives (parents, children, brothers, and sisters) give a 50% chance of having the same mutation. More distant relatives (cousins, uncles, and aunts) also have a chance of having the mutation that runs in the family. It clearly provides benefits to patients in that it allows the patient and physician to take prophylaxis approach by knowing the test result. Preventive drug therapies may then be implemented along with increased surveillance.

For breast cancer risk management, tamoxifen has been proven to cut in half the risk for women with BRCA mutations^[13]. Prophylactic bilateral mastectomy has been shown to reduce breast cancer risk by greater than 90 percent in women with a BRCA mutation or a family history of the disease^[14]. The administration of prophylactic tamoxifen therapy, pre-menopausal bilateral prophylactic oophorectomy (PO), prophylactic contralateral mastectomy (PCM) have also shown various level of improvement in life expectancy for women with unilateral breast cancer and a BRCA1 or BRCA2 gene mutation^[15].

For ovarian cancers, the risk reduction associated with greater than 5 years of oral contraceptive use is estimated in one study to be 37% to 60%^[16,17]. Prophylactic bilateral oophorectomy has been shown to reduce ovarian cancer risk by 96 percent in women with a BRCA mutation^[23].

Women with germline mutations in BRCA1 or BRCA2 have up to an 85% lifetime risk of breast cancer and up to a 46% lifetime risk ovarian cancer. Similarly, women with mutations in the DNA mismatch repair genes, MLH1, MSH2 or MSH6, associated with the Lynch/Hereditary Non-Polyposis Colorectal Cancer (HNPCC) syndrome (see below), have up to a 40–60% lifetime risk of both endometrial and colorectal cancer as well as a 9–12% lifetime risk of ovarian cancer. Genetic risk assessment enables physicians to provide individualized evaluation of the likelihood of having one of these gynecologic cancer predisposition syndromes, as well as the opportunity to provide tailored screening and prevention strategies such as surveillance, chemoprevention, and prophylactic surgery that may reduce the morbidity and mortality associated with these syndromes. Hereditary cancer risk assessment is a process that includes assessment of risk, education and counseling conducted by a provider with expertise in cancer genetics, and may include genetic testing after appropriate consent is obtained.

3. Breast Cancer Recurrence and Treatment Planning Tests

One of the latest successes of genomics test in breast cancer area is the Oncotype DX test. It is a 21-gene panel RT-PCR assay^[22, 23] and can provide information about the likelihood of systemic disease recurrence. It is important in weighing the benefits vs. the risks of adjuvant chemotherapy in order to determine the most appropriate treatment strategy – increasing confidence that the treatment plan is tailored to the individual patient. Patients with tumors that had low Recurrence Scores (RS < 18) derived minimal, or no benefit from chemotherapy. Patients with tumors that had high Recurrence Scores (RS = 31) had a large absolute benefit from chemotherapy.

The latest addition to the arsenals is the multi-gene panel test, MammaPrint, based on the well-known Amsterdam 70-gene breast cancer gene signature^[19]. In February - 2007, FDA cleared the MammaPrint test for marketing in the U.S. for node negative women under 61 years of age with tumors of less than 5cm. MammaPrint is a microarray analysis and the result classifies analyzed tumors as low or high risk for recurrence of the disease. There is only a single gene overlap between the Oncotype DX panel and the MammaPrint® panel. The reason for such diversity between gene panels under investigation is because of differences in tissue preparation, differences in laboratory methodologies, and differences in measurement techniques. At present, these gene-expression profile tests have only been validated on stored sample tissue. Whether one of the existing panels will prove to be signifi-

cantly superior to others, or whether newer panels will emerge that have better predictive power remains to be seen.

There are a number of important issues around this type of testing. First of all, gene-expression profile tests for breast cancer prognosis have been used only for specific tumor types. For example, MammaPrint® has been developed for early stage (Stage I or II) breast cancer tumors that have not metastasized to the axillary nodes, and Oncotype DX has been developed for early stage, ER+ tumors that have not metastasized to the nodes.

It is clear that additional validation studies, prospective, randomized clinical trials, and more clinical experience are needed to establish the reliability of gene expression profiling for predicting tumor recurrence and response to specific treatments. Clinical studies, such as the TAILORx trial for Oncotype DX treatment guidance^[25] and MINDACT (Microarray for Node negative Disease may Avoid ChemoTherapy)^[23] to validate the molecular markers used in MammaPrint®, are in progress to compare risk assessment profiles of node-negative breast cancer patients for adjuvant chemotherapy.

4. Breast Cancer Metastasis Detection

In addition to disease susceptibility and drug selection testing, molecular devices are becoming available for breast cancer metastasis detection. The GeneSearch BLN test is a qualitative in vitro diagnostic test for the rapid detection of metastases larger than 0.2 mm in nodal tissue removed from sentinel lymph node biopsies of breast cancer patients. It is a real time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) assay using the Cepheid SmartCycler® System that detects the presence of breast tumor cell metastasis in lymph nodes through the detection of gene expression markers, Mammaglobin (MG) and Cytokeratin 19 (CK19), that are present in higher levels in breast tissue, but not in nodal tissue (cell type specific messenger RNA)^[28].

In the test development (Cutoff Study) using 306 patients, the test performance characteristics were established as the following: sensitivity 82.4%, specificity 96.3%, PPV 89.7% and NPV 93.4%, by comparing to the standard Overall Histology. The prevalence rate of SLN was found to be at 30.6%. These parameters were confirmed by a larger validation cohort (Pivotal Study with 423 patients)^[28]. Sometimes the performance was better in the pivotal study, most likely attributed to the experience gained by the operators. As for the result interpretation, an assay result used intra-operatively that was false negative compared to later positive permanent section histology results would mean that any necessary additional axillary lymph node dissection would be performed in a second surgery rather than during the surgery for SLN biopsy. When a patient was tests assay negative and histologically detectable metastatic cancer is found later, that patient will typically require a second surgical procedure for complete axillary node dissection. The risk of the false negative is that necessary axillary lymph node

dissection (ALND) was not performed during sentinel lymph node surgery and therefore patient recalled for second operation. Permanent section histology detects nodal metastases in sentinel nodes 1-3 days after sentinel lymph node procedure. It should be noted, however, that permanent section histology itself conducted following current guidelines^[29] is reported to have false negative rates of 9-12.7%^[30,31].

5. Prostate Cancer Susceptibility Test

Prostate cancer is the most common cancer for men as breast cancer is the most common form of cancer for women. The early screening molecular test using serum samples has been developed for the prostate specific antigen (PSA) since about a quarter of a century. Although widely used for prostate cancer screening at a low cost, this test creates more controversy than any other knew molecular tests. It also has very low performance metrics in the measurement of sensitivity and specificity. Only 25 to 30 percent of men who have a biopsy due to elevated PSA levels actually have prostate cancer^[26]. The biggest concern is the uncertainty that surrounds a raised PSA level and the complications from unnecessary treatment such as surgery or radiation.

In the early 2000's, the genetic test, called uPM3, which uses urine samples, becomes available. The uPM3(TM) is based on PCA3, a specific gene that is profusely expressed in prostate cancer tissue (on average, 34 times greater than in benign prostate tissue). No other human tissues have ever been shown to produce PCA3.

The uPM3 test predicts cancer in prostate biopsy with 81% accuracy, compared to 47% accuracy for PSA. Multiple studies reported at several urology meetings throughout 2002 and 2003 have confirmed that the test helps urologists solve the significant diagnostic dilemma of men who have an elevated PSA and a negative biopsy, but who are strongly suspected of having prostate cancer.

In 2006, the prototype test, Aptima PCA3^[27] and the next generation PCA3 test, PCA3Plus, as a successor of the uPM3 test, becomes available at an even higher sensitivity of 95.7%.

6. Colorectal Cancer Screen Test and Susceptibility Test

Colorectal cancer (CRC) is the third most common cancer and third leading cause of cancer death in the United States. When found early, the disease can be prevented or easily treated. The gene mutation detection test has been made available since 1990's. The first DNA sequencing test based on the mutations in MLH1, MSH2, or MSH6 genes was offered by Myriad Genetics. The latest development and commercial availability of stool DNA based screening test is offered by Exact Sciences^[44]. According to the American Cancer Society, only 37% of all new cases of colorectal cancer are detected in the early stages when the disease is 90% curable. Many patients diagnosed in the late stages had never received regular

screening and presented with no symptoms. Stool-based DNA screening can help to be an effective tool for eliminating the barriers to screening for one of the most curable cancers in our society. The stool DNA screening test offers the hope for early detection of the gene mutations in the CRC tumor patients [22].

In a 5,400 patient study, using a direct comparison between stool-based DNA screening for colon cancer and FOBT (Hemoccult II), stool-based DNA screening demonstrated an overall sensitivity of 65% - four times greater than FOBT for asymptomatic, average-risk individuals 50 years of age or older, while specificity maintained at 95% level.

Unlike bleeding, which can be intermittent or non-existent, DNA exfoliation occurs continuously and exfoliated cells are representative of the entire colon.

Also, unlike bleeding, an indirect measure that is not associated with the underlying etiology of colorectal cancer, DNA alterations such as those in stool based DNA screening are specifically linked to the cause and actual presence of cancer and pre-malignant polyps. [21]

Summary remarks - accelerated pace of personalized diagnostics development and adoption in the clinical practices

The combination of technology and genomic research activities produce tremendous amount of new insights into the clinical samples and data collected over the history of medical researches. For example, the recent genome-wide association studies using SNP genotyping techniques of Affy100K chips on 17 phenotypes and biomarkers have generated unprecedented knowledge about coronary artery diseases, blood pressure, arrhythmia, cancers, diabetes, cognitive capacity, osteoporosis, longevity, and atherosclerosis from the Farmingham cohort.

Continued technology innovation and improvement is another driver to the rapid laboratory development and the user-friendly point-of-care delivery of the molecular diagnostic tests. Luminex's xMap, Cepheid's GeneXpert are a few of such platforms that are available today and more competing platforms are in the pipeline to be ready for considerations by the clinical laboratories for industrial applications.

Even though the necessary technologies to develop personalized diagnostic tests are available, barriers such as the expense of clinical trials and difficulty obtaining clinical samples have significantly slowed the development process. The venture capitalists and investors have come to the realization of the opportunity and start to capitalize on this. The industry, academic and investor multi-party partnership will ensure focused and accelerated progress on the development, testing and validation of new molecular diagnostic tools and the approval and distribution of these tools for widespread clinical use.

On the test development side, the regulatory pathways are typically through CLIA (Clinical Laboratory Improvement Act 1988) regulations. CLIA is a federal legislation enforced at the state level with some states requiring extra items of details for qualification of management and technical personnel of the clinical laboratories. This is administered by the CMS. Recently, however, FDA has made major effort in capturing the responsibility by issuing a couple of guideline documents on biomarker and pharmacogenomics in drug and device development, IVDMLA and companion diagnostics tests. A major regulatory paradigm shift is on the horizon for the molecular testing industry.

On the clinician side, the pace of adoption of the new test for disease diagnosis, patient risk stratification and personalized therapies is accelerating as well. For example, the Society of Gynecologic Oncologists has recently released their statement on guidelines for risk assessment for inherited cancer predispositions.

Finally, the health economics issues, the pricing and reimbursement issues, the ethical issues, and societal issues need to be considered for the genetic testing to be widely accepted.

References:

1. Sliwkowski MX, Lofgren JA, Lewis GD, Hotaling TE, Fendly BM, Fox JA. Nonclinical studies addressing the mechanism of action of trastuzumab (Herceptin). *Semin Oncol.* 1999;26:60-70.
2. Yakes FM, Chinratanalab W, Ritter CA, King W, Seelig S, Arteaga CL. Herceptin-induced inhibition of phosphatidylinositol-3 kinase and Akt is required for antibody-mediated effects on p27, cyclin D1, and antitumor action. *Cancer Research.* 2002;62:4132-4141.
3. Arnould L, Gelly M, Penault-Llorca F. Trastuzumab-based treatment of HER2-positive breast cancer: an antibody-dependent cellular cytotoxicity mechanism? *Br J Cancer.* 2006;94:259-267.
4. Tsuda H, Akiyama F, Terasaki H, Hasegawa T, Kurosumi M, Shimadzu M, et al. Detection of HER-2/neu (c-erb B-2) DNA amplification in primary breast carcinoma. Interobserver reproducibility and correlation with immunohistochemical HER-2 overexpression. *Cancer* 2001;92:2965-74.
5. Persons DL, Bui MM, Lowery MC, Mark HFL, Yung J-F, Birkmeier JM, et al. Fluorescence in situ hybridization (FISH) for detection of HER-2/neu amplification in breast cancer: a multicenter portability study. *Ann Clin Lab Sci* 2000;30:41-8.
6. Hayes, DF, et al. for Cancer and Leukemia Group B (CALGB) Investigators: HER2 and Response to Paclitaxel in Node-Positive Breast Cancer. *New Engl. J. Med.* 2007; 357:1496-1506.

7. Castilla LH, Couch FJ, Erdos MR, Hoskins KF, Calzone K, Garber JE, Boyd J, Lubin MB, Deshano ML, Brody LC, et al.: Mutations in the BRCA1 gene in families with early-onset breast and ovarian cancer. *Nat Genet.* 1994 Dec;8(4):387-91.
8. Thompson ME, Jensen RA, Obermiller PS, Page DL, Holt JT: Decreased expression of BRCA1 accelerates growth and is often present during sporadic breast cancer progression. *Nat Genet.* 1995 Apr;9(4):444-50.
9. Chen Y, Chen CF, Riley DJ, Allred DC, Chen PL, Von Hoff D, Osborne CK, Lee WH: Aberrant subcellular localization of BRCA1 in breast cancer. *Science.* 1995 Nov 3;270(5237):789-91.
10. Wooster, R.; Neuhausen, S. L.; Mangion, J.; Quirk, Y.; Ford, D.; Collins, N.; Nguyen, K.; Seal, S.; Tran, T.; Averill, D.; Fields, P.; Marshall, G. et al.: Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. *Science* 1994; 265: 2088-2090.
11. Wooster, R.; Bignell, G.; Lancaster, J.; Swift, S.; Seal, S.; Mangion, J.; Collins, N.; Gregory, S.; Gumbs, C.; Micklem, G.; Barfoot, R.; Hamoudi, R. et al.: Identification of the breast cancer susceptibility gene BRCA2. *Nature* 1995; 378: 789-792.
12. Breast Cancer Linkage Consortium : Pathology of familial breast cancer: differences between breast cancers in carriers of BRCA1 and BRCA2 mutations and sporadic cases. *Lancet* 1997; 349: 1505-1510.
13. Vogel VG.: Recent results from clinical trials using SERMs to reduce the risk of breast cancer. *Ann N Y Acad Sci.* 2006 Nov;1089:127-42.
14. Metcalfe, KA: Prophylactic Bilateral Mastectomy for Breast Cancer Prevention. *J. Women's Health.* 2004, 13(7): 822-829.
15. Schrag D, Kuntz KM, Garber JE, Weeks JC: Life expectancy gains from cancer prevention strategies for women with breast cancer and BRCA1 or BRCA2 mutations. *JAMA.* 2000 Feb 2;283(5):617-24.
16. Narod, SA. et al. for The Hereditary Ovarian Cancer Clinical Study Group: Oral Contraceptives and the Risk of Hereditary Ovarian Cancer. 1998; 339:424-428.
17. National Institutes of Health: Consensus Development Conference Statement on Ovarian Cancer: Screening, Treatment, and Followup. April 5-7, 1994. <http://consensus.nih.gov/1994/1994OvarianCancer096html.htm>.
18. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, Baehner FL, Walker MG, Watson D, Park T, Hiller W, Fisher E, Wickerham DL, Bryant J, Wolmark N.: A Multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. (GHI/NSABP Study B-14). *New England Journal of Medicine* 2004;351(27):2817-2826.
19. Meigs JB, Manning AK, Fox CS, Florez JC, Liu C, Cupples LA, Dupuis J: Genome-wide association with diabetes-related traits in the Framingham Heart Study. *BMC Med Genet.* 2007 Sep 19;8 Suppl 1:S16.
20. Murabito JM, Rosenberg CL, Finger D, Kreger BE, Levy D, Splansky GL, Antman K, Hwang SJ: A genome-wide association study of breast and prostate cancer in the NHLBI's Framingham Heart Study. *BMC Med Genet.* 2007 Sep 19;8 Suppl 1:S6.
21. EXACT Sciences:
22. Loktionov et al.: Quantification of DNA From Exfoliated Colonocytes Isolated From Human Stool Surface as a Novel Noninvasive Screening Test for Colorectal Cancer. *Clinical Cancer Research* 1998;4:337-42.
23. Lancaster, JM et al. for Society of Gynecologic Oncologists Education Committee: Statement on Risk Assessment for Inherited Gynecologic Cancer Predispositions. *Gynecologic Oncology* 107 (2007) 159–162. Also Available online at www.sciencedirect.com.
24. Olopade OI, Artioli G.: Efficacy of risk-reducing salpingo-oophorectomy in women with BRCA-1 and BRCA-2 mutations. *Breast J.* 2004 Jan-Feb;10 Suppl 1:S5-9.
25. Sparano, JA.: The TAILORx trial: individualized options for treatment. *Commun Oncol* 2006;3:494–496.
26. Keetch DW, Catalona WJ, Smith DS. Serial prostatic biopsies in men with persistently elevated serum prostate specific antigen values. *The Journal of Urology* 1994; 151(6):1571–1574.
27. Fradet Y, Groskopf J, Walker S, et al. Prototype Aptima PCA3 molecular urine test: development of a method to aid in the diagnosis of prostate cancer. Program and abstracts of the American Urological Association 2006 Annual Meeting; May 20-25, 2006; Atlanta, Georgia. Abstract 538.
28. FDA web site: GeneSearch Test Kit Approval letter and label (2007): <http://www.fda.gov/cdrh/pdf6/p060017c.pdf>
29. Yared, MA, Middleton, LP, Smith TL, et al. Recommendations for sentinel lymph node processing in breast cancer. *Am J Surg Pathol* 2000; 26(3): 377-382.
30. Treseler, PA and Tauchi, P. S. Pathologic Analysis of the Sentinel Lymph Node. *Surg Clin North Am.* 2000;Dec 80(6): 1695-719.
31. Whitworth P, McMasters KM, Tafra L, et al. State-of-the-art lymph node staging for breast cancer in the year 2000. *Am J Surg* 2000; 180:262-267.