The Practical Use of Molecular Profiling

Written by ***Emma Hitt, PhD***

This session explored the use of molecular profiling in three stages of breast cancer development: cacinoma in situ, primary, and metastatic. The treatment of in situ carcinoma of the breast is controversial. In the molecular progression from normal tissue to invasion, in situ carcinoma is a late-occurring step associated with an increased risk of the subsequent development of invasive carcinoma [Bombonati A, Sgroi DC. *J Pathol* 2011]. Lawrence J. Solin, MD, Einstein Medical Center, Philadelphia, Pennsylvania, USA, presented the currently available data on molecular profiling of in situ carcinoma of the breast.

Predictors of low-risk ductal carcinoma in situ (DCIS) are poorly defined using clinical and pathologic characteristics. Randomized trials of radiation and tamoxifen have shown a reduced risk for patients with DCIS. Molecular profiling for DCIS may shed light into its underlying tumor biology, potentially allowing for improved risk assessment and individualized treatment decisions.

During the same session, Antonio C. Wolff, MD, The Johns Hopkins Kimmel Comprehensive Cancer Center, Baltimore, Maryland, USA, provided an overview of molecular profiling of breast cancer. From 1980 to 2005, decisions about adjuvant chemotherapy were based on anatomy, nodal status, and tumor size, and adjuvant endocrine treatment was given if the tumor was estrogen receptor (ER)-positive. Since 2005, there has been a greater focus on stratifying breast cancer therapy decisions according to the biologic subtypes, including ER-positive/human epidermal growth factor receptor 2 (HER2)-negative, HER2-positive, and triple-negative breast cancer. While these phenotypes inform the choice of therapy, they do not offer predictive information on which patients will benefit from the treatment.

The American Society of Clinical Oncology (ASCO) tumor marker guidelines for breast cancer recommends measuring ER and progesterone receptor (PR) on primary invasive breast cancers to determine which patients are candidates for endocrine therapy [Harris L et al. *J Clin Oncol* 2007] and evaluate HER2 expression to guide selection of trastuzumab therapy. While adjuvant endocrine therapy is standard of care in patients with tumors that express ER and/or PR, the indication for adjuvant chemotherapy is less clear-cut. It is well established that ER-positive breast tumors derive less benefit from chemotherapy compared with ER-negative tumors [Berry DA et al. *JAMA* 2006].

The ASCO guidelines suggest use of the 21-gene Oncotype DX recurrence score (RS) assay to help identify subsets of patients with ER-positive breast cancers who may benefit from the addition of chemotherapy to endocrine therapy. Another prognostic multigene signature that may have utility in predicting for adjuvant chemotherapy benefit is the FDA-approved 70-gene MammaPrint signature. Unlike the 21-gene Oncotype RS where the genes were preselected, MammaPrint was developed using an unsupervised hierarchical clustering approach whereby the high-risk gene signature predicted for poor outcomes in tumors of all subtypes. Both these assays were tested retrospectively; however, the Oncotype RS was evaluated retrospectively in a prospectively assembled clinical trial (Table 1). Both these multigene signatures are currently undergoing prospective validation in large ongoing studies. The TAILORx trial [NCT00310180] will study the utility of the 21-gene RS signature to predict for chemotherapy benefit in the intermediate score range, while the MINDACT trial [NCT00433589] will study the outcomes of patients with discordant risk assessments when using the 70-gene signature and clinicopathologic features using the Adjuvant! Online program.

Lajos Pusztai, MD, DPhil, Yale Cancer Center, New Haven, Connecticut, USA, reported on the current use of genomic and molecular tests in routine practice and clinical research in metastatic breast cancer (MBC). In the management of MBC, there is no genomic test that is used routinely at this time. Measuring circulating tumor cells may help assess prognosis of MBC, but this currently has limited impact on the choice of therapy.

Discordance between HER2 or ER/PR receptor status in primary and metastatic lesions has been reported as 15% to 30%, depending on the study. This discordance—due to both technical reasons and true biologic changes in the cancer—is expected. It is not possible at present to distinguish between these two causes or to accurately estimate their contribution to differences in receptor status. [Pusztai L et al. *Oncologist* 2010]. Clinicians could consider repeating HER2 and/or ER/PR measurements on the metastatic tumors, if possible, as this may potentially aid treatment decisions.

Several genomic-based tests are being evaluated as predictive markers of therapeutic efficacy in MBC. These include the development of gene expression signatures to predict chemotherapy response, the identification

of genomic abnormalities that have been successfully targeted in other cancers, and the development of gene predictors based on drug mechanism-of-action.

Molecular markers are being increasingly used in clinical trials for patient selection criteria or as an enrichment development. Targetable abnormalities appear to be individually rare in breast cancer, however they are present in up to 50% of breast cancers collectively. Due to low marker prevalence, Dr. Pusztai proposed that multiple tests should be performed at once and the results should be used to triage patients to targeted therapies. The Yale Molecular Analysis Prior to Investigational Therapy program is currently being conducted in a series of Phase 2 trials. These are biomarker-driven adaptive trials comparing the outcomes of patients with MBC with and without a specific gene mutation treated with specific targeted therapies. Dr. Pusztai concluded, "One of the most important future challenges is to design experimental and informatics tools that could guide how to combine targeted agents to match the multiple abnormalities that individual cancers have."

Table 1. Commercially Available Prognostic Multigene Signatures in Breast Cancer.

	MammaPrint	Veridex 76-Gene*	MapQuant Dx/ Simplified	Oncotype DX	Breast Cancer Index (HoxB13:IL17BR/MGI)
Analysis	Microarray	Microarray	Microarray/qRT-PCR	qRT-PCR	qRT-PCR
Provider	Agendia (Amsterdam, Netherlands)	Currently not available	Ipsogen (Marseille, France)	Genomic Health (Redwood City, CA, USA)	bioTheranostics (San Diego, CA, USA)
Assay	70-gene signature	76-gene signature	97-gene signature or eight-gene PCR	21-gene recurrence score	Two-gene HOXB13:IL17R/ molecular grade index
Tissue type	Frozen or stabilised mRNA	Frozen	Frozen or FFPE	FFPE	FFPE
Discovery set	78 ER±, N0, <5 cm diameter cancers, age <55 years	115 ER±, N0 cancers	64 ER+ cancers	447 ER+ samples, including samples from the tamoxifen-only group of the NSABP B-20 trial	60 ER+ tumors, tamoxifen only treated patients 20 microdissected FFPE samples
Initial validation set	295 ER±, N±, <5 cm diameter cancer, age <52 years	171 ER±, N0 cancers	597 ER± cancers, of which 125 profiled in-house	668 ER+ samples from NSABP B-14 trial (tamoxifen-treated)	20 ER+ FFPE samples
Outcome	Distant metastasis at 5 years	Distant metastasis at 5 years	Good (GGI I) or poor (GGI III) prognosis	Disease-free relapse at 10 years	Relapse-free and overall survival
Clinical application	Prognosis of N0, <5 cm diameter, stage I/II disease, age <61 years	Prognosis of N0 patients	Molecular grading, for ER+, histological grade II disease	Prediction of recurrence risk in ER+ and N0 disease treated with tamoxifen	Prognostic in ER+ disease, prediction of response to tamoxifen
Results presentation	Dichotomous; good or poor prognosis	Dichotomous; good or poor prognosis	Dichotomous, GGI I or GGI III	Continuous variable; recurrence score	Continuous variable; risk of recurrence score
Additional information provided	mRNA levels of ER, PR, and HER2 (Targetprint) Intrinsic subtypes (Blueprint)	..	..	mRNA levels of ER, PR, and HER2	Molecular grade index
Prognostic value in other populations	Up to 3 positive nodes, and HER2+ disease	ER+, N0 patients treated with tamoxifen	ER+ receiving aromatase inhibitors	ER+ and 1–3 N+, ER+ postmenopausal receiving aromatase inhibitors	..
Predictive value	Chemotherapy response (poor prognosis group)	Chemotherapy response (poor prognosis group)†	Chemotherapy response (GGI III)	Chemotherapy response (high recurrence score)	Chemotherapy response (high risk of recurrence score)†
Level of evidence	II	III	III	I	III
FDA approval	Yes	No	No	No	No

±=positive and negative; ER=estrogen receptor; FFPE=formalin-fixed paraffin-embedded; GGI=genomic grade index; MGI=molecular grade index; N=lymph nodes; PR=progesterone receptor; qRT-PCR=quantitative reverse transcription-polymerase chain reaction. *Veridex is not currently commercially available. †Hypothetical use, based on indirect evidence.
Reprinted from *The Lancet*, Vol. 3078. Reis-Filho JS, Pusztai L. Gene Expression Profiling in Breast Cancer: Classification, Prognostication, and Prediction. 1812-23. Copyright 2011, with permission from Elsevier.