Short communication
Markers of endocrine sensitivity
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There are few markers for which there is a sufficiently high level of evidence to justify their use in clinical decision making. In order to reach such levels, the evidence must address a specific utility, such as prognosis or prediction, so that the outcome of a patient in one state designated by the marker (for example, a patient with a negative test result) would be so different from that in another patient in a different state (for example, a patient with a positive test result) that she (or he) would accept a different treatment recommendation. Many assume that a marker is used to determine whether to treat a patient with a given therapeutic agent. However, and especially with regard to treatments with relatively low toxicity and high impact such as endocrine treatments, the question is usually 'Will there be so little benefit from the treatment that she is willing to forego this therapy to avoid toxicities?' The level of evidence that would drive use of a marker must be such that estimates of outcome differences are reliable. Reliable estimates are a function of rigorous technical development to ensure analytical accuracy, appropriate study design to address the chosen utility, and sophisticated data analysis (including independent validation) so that one may be certain that the confidence limits of the estimates are narrow.

Endocrine therapy has been a mainstay of treatment of breast cancer since the late 1800s [1], and it has led to remarkable palliation, mortality reduction and even prevention in women with or at risk for this disease. A breast cancer can be either oestrogen independent (and therefore refractory to all endocrine treatments) or endocrine dependent but resistant to specific endocrine strategies and even to specific agents. Because endocrine therapy is expensive and may be associated with frequent bothersome and occasionally life-threatening toxicities, a marker of either absolute endocrine independence or resistance to specific therapies would be remarkably valuable in caring for women with breast cancer.

Of course, the oestrogen receptor (ER) represents such a marker. ER was first identified by Jensen and colleagues [2]. Very soon afterward, McGuire and colleagues [3-5] showed that although prediction of resistance was not absolute, women with ER-negative or low metastatic breast cancers were very unlikely to respond to a variety of anti-oestrogen therapies. Subsequent meta-analyses conducted by the Early Breast Cancer Trialists' Collaborative Group (the Oxford Overview) [6] confirmed nearly complete lack of benefit from adjuvant tamoxifen in ER-negative patients. Indeed, ER is one of the few tumour markers recommended by the Tumor Marker Guidelines Committee of the American Society of Clinical Oncology for routine use in the evaluation and treatment of patients with breast cancer [7].

Although it is highly unlikely that patients with ER-negative tumours will benefit from endocrine treatment, only 50% to 60% of those with ER-positive, or rich, breast cancers will. This observation raises a critical question regarding the technical and biological accuracy of ER measurement. Early assays of ER were performed using ligand (oestradiol)-binding assays (LBAs), which are technically difficult, require relatively large amounts of fresh, frozen tissue, and can be complicated by prolonged delays to freezing and variable amounts of cancer within the tissue. In the late 1980s, polyclonal and monoclonal antibodies to ER became available, permitting immunohistochemical evaluation of ER in fixed tissue with *in situ* assessment of whether the cancer cells themselves were positive or negative. However, surprisingly, the ability of immunohistochemistry to predict benefit from various endocrine therapies has never been as well vetted as that of the LBAs. Rather, correlative studies demonstrating relative immunohistochemical scores with LBA results were reported, and a variety of issues regarding the technical components of immunohistochemistry and correlation with clinical outcomes have never been addressed properly. Indeed, the American Society of Clinical Oncology

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AI = aromatase inhibitor; ER = oestrogen receptor; HER = human epidermal growth factor receptor; LBA = ligand (oestradiol)-binding assay; PR = progesterone receptor; RS = recurrence score; RT-PCR = reverse transcription polymerase chain reaction; SNP = single nucleotide polymorphism.

has partnered with the College of American Pathologists to establish guidelines and proficiency testing for evaluation of another critical marker, human epidermal growth factor receptor (HER)2 [8], and a similar initiative is planned for ER in the future.

A variety of candidate markers have been proposed that might complement and further refine the predictive utility of ER. To start, simple quantitative analysis of ER might provide additional information. Indeed, in protocol B14, in which patients were randomly assigned to adjuvant tamoxifen or placebo, the National Adjuvant Bowel and Breast Project reported a stepwise additional benefit from increasing deciles of ER content when measured by LBA (S Paik, personal communication). However, even patients with very low ER levels seem to benefit from tamoxifen [9]. In addition to ER, other potential factors include ER-β, progesterone receptor (PR), ER co-activating and repressing proteins, the epithelial growth factor receptor family, and various markers of cell survival and proliferation. Of these, perhaps PR and HER2 are the most intensively studied. Preclinical and preliminary clinical studies strongly suggested that absence of PR and/or elevated expression of HER2 are associated with either relative resistance to all endocrine therapies or specific resistance to certain types (such as selective ER modulators, for example tamoxifen) but ongoing sensitivity to other strategies (such as oestrogen depletion with aromatase inhibitors [AIs] in postmenopausal women). To summarize a great deal of literature, it is not clear that either marker contributes to decision making regarding endocrine treatment in women with ER-positive breast cancer. Although low PR and high HER2 levels are consistently associated with worse prognosis, neither of these conditions preclude endocrine treatment in a patient with ER-positive breast cancer, and results regarding selection of tamoxifen versus an AI with these markers have been inconsistent [10-14].

PR may be helpful in selecting ER-negative patients who might benefit from tamoxifen [10]. ER-negative/PR-positive tumours are uncommon, and this utility remains controversial. Nonetheless, it seems prudent to recommend endocrine treatment in this setting, in order to avoid under-treatment with such a highly effective and relatively low toxicity strategy.

Recent technological advances have permitted analysis of expression of several genes simultaneously, resulting in a 'profile' or 'signature' pattern [15]. Although several of these appear to provide prognostic information, only a few have been tested specifically to determine their clinical utility as a predictor of outcome in patients treated with endocrine therapy [16,17]. Of these, the 21-gene recurrence score (RS; OncotypeDX, Genomics Health Inc., Redwood City, CA, USA) incorporates semi-quantitative analysis of ER and downstream genes, HER2 and associated genes, a number of proliferation genes, and selected genes that are ostensibly related to cell survival and metastatic potential, using a multiparameter assay based on RT-PCR [18]. Using archived, formalin-fixed, paraffin-embedded tissues, the National Surgical Adjuvant Breast and Bowel Project investigators have reported that approximately 50% of patients with node-negative, ER-positive (as determined by LBA) breast cancer treated with only tamoxifen have low RS. These patients have a remarkably favourable prognosis over 10 years, whereas the remaining 50% of patients fall into the intermediate or high RS categories, with consequent less favourable outcomes [17,19]. Moreover, in exploratory analyses this assay appeared to be predictive of benefit from tamoxifen (low RS predicts benefit and high RS predicts resistance) and from chemotherapy (low RS predicts resistance and high RS predicts benefit) [19,20].

Finally, recently reported results have suggested that inherited, germ-line factors may also explain differential benefit and toxicity between patients treated with endocrine therapies. In particular, inherited single nucleotide polymorphisms (SNPs) in *CYP2D6* result in poor conversion of the relatively inactive parent compound, tamoxifen, into its most active metabolite, endoxifen [21-23]. Although controversial, some studies have suggested that patients with these SNPs may have a worse outcome than those with wild-type *CYP2D6* when treated with tamoxifen [24]. Likewise, SNPs in the genes that encode ER-α and ER-β (*ESR1* and *ESR2*) may also modulate the nontumoural effects of tamoxifen on lipid levels, hot flushes and bone mineral density [25,26], and SNPs in the gene that encodes aromatase (*CYP19*) may affect the activities of various AIs [27].

In summary, ER is clearly a potent and important predictive factor that should be evaluated and used, in each patient with breast cancer, to determine whether endocrine treatment is appropriate. Other putative markers of endocrine resistance in ER-positive cancers, although supported by strong preclinical data, have not yet achieved a sufficient level of evidence that one should withhold potentially life-saving and beneficial therapy based on their results.

Competing interests

Acknowledgement

References

1. Beatson GW: **On the treatment of inoperable cases of carcinoma of the mamma: Suggestions for a new method of treatment with illustrative cases.** *Lancet* 1896, **148:**104-107.
2. De Sombre ER, Puca GA, Jensen EV: **Purification of an estrophilic protein from calf uterus.** *Proc Natl Acad Sci USA* 1969, **64:**148-154.

3. McGuire WL: **Estrogen receptors in human breast cancer.** *J Clin Invest* 1973, **52:**73-77.
4. McGuire W, Carbone PP, Sears ME, *et al*.: **Estrogen receptors in human breast cancer.** In *Estrogen Receptors in Human Breast Cancer.* Edited by McGuire WL, Carbone PP, Vollmer EP. New York City, NY: Raven Press; 1975:1-7.
5. Osborne CK, McGuire WL: **Current use of steroid hormone receptor assays in the treatment of breast cancer.** *Surg Clin North Am* 1978, **58:**777-788.
6. Early Breast Cancer Trialists' Collaborative Group: **Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials.** *Lancet* 2005, **365:**1687-1717.
7. Harris L, Fritsche H, Mennel R, Norton L, Ravdin P, Taube S, Somerfield MR, Hayes DF, Bast RC Jr: **American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer.** *J Clin Oncol* 2007, **25:**5287-5312.
8. Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM, Hayes DF; American Society of Clinical Oncology; College of American Pathologists: **American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer.** *J Clin Oncol* 2007, **25:**118-145.
9. Harvey JM, Clark GM, Osborne CK, Allred DC: **Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer.** *J Clin Oncol* 1999, **17:**1474-1481.
10. Osborne CK, Yochmowitz MG, Knight WA III, McGuire WL: **The value of estrogen and progesterone receptors in the treatment of breast cancer.** *Cancer* 1980, **46(suppl):**2884-2888.
11. Ravdin PM, Green S, Dorr TM, McGuire WL, Fabian C, Pugh RP, Carter RD, Rivkin SE, Borst JR, Belt RJ, *et al*: **Prognostic significance of progesterone receptor levels in estrogen receptor-positive patients with metastatic breast cancer treated with tamoxifen: results of a prospective Southwest Oncology Group study.** *J Clin Oncol* 1992, **10:**1284-1291.
12. Rasmussen BB, Regan MM, Lykkesfeldt AE, Dell'Orto P, Del Curto B, Henriksen KL, Mastropasqua MG, Price KN, Méry E, Lacroix-Triki M, Braye S, Altermatt HJ, Gelber RD, Castiglione-Gertsch M, Goldhirsch A, Gusterson BA, Thürlimann B, Coates AS, Viale G; BIG 1-98 Collaborative and International Breast Cancer Study Groups: **Adjuvant letrozole versus tamoxifen according to centrally-assessed ERBB2 status for postmenopausal women with endocrine-responsive early breast cancer: supplementary results from the BIG 1-98 randomised trial.** *Lancet Oncol* 2008, **9:**23-28.
13. Viale G, Regan MM, Maiorano E, Mastropasqua MG, Golouh R, Perin T, Brown RW, Kovács A, Pillay K, Ohlschlegel C, Braye S, Grigolato P, Rusca T, Gelber RD, Castiglione-Gertsch M, Price KN, Goldhirsch A, Gusterson BA, Coates AS: **Chemoendocrine compared with endocrine adjuvant therapies for node-negative breast cancer: predictive value of centrally reviewed expression of estrogen and progesterone receptors - International Breast Cancer Study Group.** *J Clin Oncol* 2008, **26:**1404-1410.
14. Dowsett M, Allred C, Knox J, Quinn E, Salter J, Wale C, Cuzick J, Houghton J, Williams N, Mallon E, Bishop H, Ellis I, Larsimont D, Sasano H, Carder P, Cussac AL, Knox F, Speirs V, Forbes J, Buzdar A: **Relationship between quantitative estrogen and progesterone receptor expression and human epidermal growth factor receptor 2 (HER-2) status with recurrence in the Arimidex, Tamoxifen, Alone or in Combination trial.** *J Clin Oncol* 2008, **26:**1059-1065.
15. Van't Veer LJ, Paik S, Hayes DF: **Gene expression profiling of breast cancer: a new tumor marker.** *J Clin Oncol* 2005, **23:**1631-1635.
16. Goetz MP, Suman VJ, Ingle JN, Nibbe AM, Visscher DW, Reynolds CA, Lingle WL, Erlander M, Ma XJ, Sgroi DC, Perez EA, Couch FJ: **A two-gene expression ratio of homeobox 13 and interleukin-17B receptor for prediction of recurrence and survival in women receiving adjuvant tamoxifen.** *Clin Cancer Res* 2006, **12:**2080-2087.
17. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, Baehner FL, Walker MG, Watson D, Park T, Hiller W, Fisher ER, Wickerham DL, Bryant J, Wolmark N: **A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer.** *N Engl J Med* 2004, **351:**2817-2826.
18. Cronin M, Pho M, Dutta D, Stephans JC, Shak S, Kiefer MC, Esteban JM, Baker JB: **Measurement of gene expression in archival paraffin-embedded tissues: development and performance of a 92-gene reverse transcriptase-polymerase chain reaction assay.** *Am J Pathol* 2004, **164:**35-42.
19. Paik S, Tang G, Shak S, Kim C, Baker J, Kim W, Cronin M, Baehner FL, Watson D, Bryant J, Costantino JP, Geyer CE Jr, Wickerham DL, Wolmark N: **Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer.** *J Clin Oncol* 2006, **24:**3726-3734.
20. Albain K, Barlow W, Shak S, Hortobagyi G, Livingston R, Yeh IT, Ravdin P, Yoshizawa C, Baehner FL, Davidson N, *et al*.: **Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal, node-positive, ER-poisitive breast cancer (S8814, INT0100).** *Breast Cancer Res Treat* 2007, **106 (suppl 1):**late breaking abstract, #10.
21. Stearns V, Johnson MD, Rae JM, Morocho A, Novielli A, Bhargava P, Hayes DF, Desta Z, Flockhart DA: **Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine.** *J Natl Cancer Inst* 2003, **95:**1758-1764.
22. Jin Y, Desta Z, Stearns V, Ward B, Ho H, Lee KH, Skaar T, Storniolo AM, Li L, Araba A, Blanchard R, Nguyen A, Ullmer L, Hayden J, Lemler S, Weinshilboum RM, Rae JM, Hayes DF, Flockhart DA: **CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment.** *J Natl Cancer Inst* 2005, **97:**30-39.
23. Borges S, Desta Z, Li L, Skaar TC, Ward BA, Nguyen A, Jin Y, Storniolo AM, Nikoloff DM, Wu L, Hillman G, Hayes DF, Stearns V, Flockhart DA: **Quantitative effect of CYP2D6 genotype and inhibitors on tamoxifen metabolism: implication for optimization of breast cancer treatment.** *Clin Pharmacol Ther* 2006, **80:**61-74.
24. Hayes DF, Stearns V, Rae J, Flockhart D: **A model citizen? Is tamoxifen more effective than aromatase inhibitors if we pick the right patients?** *J Natl Cancer Inst* 2008, **100:**610-613.
25. Ntukidem NI, Nguyen AT, Stearns V, Rehman M, Schott A, Skaar T, Jin Y, Blanche P, Li L, Lemler S, Hayden J, Krauss RM, Desta Z, Flockhart DA, Hayes DF; Consortium on Breast Cancer Pharmacogenomics: **Estrogen receptor genotypes, menopausal status, and the lipid effects of tamoxifen.** *Clin Pharmacol Ther* 2008, **83:**702-710.
26. Henry NL, Nguyen A, Azzouz F, Li L, Robarge J, Philips S, Cao D, Skaar T, Storniolo AM, Flockhart DA, *et al*: **Association of estrogen receptor polymorphisms and change in bone mineral density with tamoxifen therapy.** *J Clin Oncol* 2008, in press.
27. Colomer R, Monzo M, Tusquets I, Rifa J, Baena JM, Barnadas A, Calvo L, Carabantes F, Crespo C, Muñoz M, Llombart A, Plazaola A, Artells R, Gilabert M, Lloveras B, Alba E: **A single-nucleotide polymorphism in the aromatase gene is associated with the efficacy of the aromatase inhibitor letrozole in advanced breast carcinoma.** *Clin Cancer Res* 2008, **14:**811-816.