Clinical Oncology recommend that postmenopausal women "consider incorporating AI therapy at some point during adjuvant treatment, either as up-front therapy or as sequential treatment after tamoxifen," limit total AI exposure to 5 years and acknowledge that the "optimal timing and duration of endocrine treatment remain unresolved."[17(p3784)] Alas, we have still not answered the pressing questions of sequence and duration. Several clinical trials are seeking to define the appropriate duration of adjuvant aromatase inhibition, and those data are awaited eagerly.

The long-term follow-up data in the articles that accompany this editorial and elsewhere[2-4,12,14] testify to the long arc of hormone receptor–positive breast cancer, confirm the enduring overall safety of tamoxifen and AIs, and provide reassurance that the well-characterized major adverse events of therapy either stabilize or resolve with cessation of treatment. The substantial near-term successes of adjuvant chemotherapy and endocrine therapy have shifted both the natural history and the dialogue in ER-positive, early-stage breast cancer for oncologists and patients alike. The issue of late recurrence—deep time for clinicians and survivors—has emerged as a fundamental challenge. RCTs have shown equivalence for either 5 years of AI treatment or a sequenced regimen of tamoxifen followed by an AI for a total of 5 years.[13,18] For women who receive AI-based adjuvant treatment, it remains unclear whether a longer program of extended therapy with an AI beyond 5 years of initial adjuvant treatment will outperform a shorter 5-year course of adjuvant endocrine therapy. Progress in the deep time problem of early-stage breast cancer will depend on answering the long and the short of that question.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTIONS

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REFERENCES

1. Early Breast Cancer Trialists' Collaborative Group (EBCTCG), Davies C, Godwin J, et al: Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: Patient-level meta-analysis of randomised trials. Lancet 378:771-784, 2011

2. Bliss JM, Kilburn LS, Coleman RE, et al: Disease-related outcomes with long-term follow-up: An updated analysis of the Intergroup Exemestane Study. J Clin Oncol 30:709-717, 2012

3. Cuzick J, Sestak I, Baum M, et al: Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 10-year analysis of the ATAC trial. Lancet Oncol 11:1135-1141, 2010

4. Regan MM, Neven P, Giobbie-Hurder A, et al: Assessment of letrozole and tamoxifen alone and in sequence for postmenopausal women with steroid hormone receptor-positive breast cancer: The BIG 1-98 randomised clinical trial at 8.1 years median follow-up. Lancet Oncol 12:1101-1108, 2011

5. Mauriac L, Keshaviah A, Debled M, et al: Predictors of early relapse in postmenopausal women with hormone receptor-positive breast cancer in the BIG 1-98 trial. Ann Oncol 18:859-867, 2007

6. Kennecke H, McArthur H, Olivotto IA, et al: Risk of early recurrence among postmenopausal women with estrogen receptor-positive early breast cancer treated with adjuvant tamoxifen. Cancer 112:1437-1444, 2008

7. Debled M, MacGrogan G, Brouste V, et al: Prognostic factors of early distant recurrence in hormone-receptor positive, postmenopausal breast cancer patients receiving adjuvant tamoxifen therapy: Results of a retrospective analysis. Cancer 109:2197-2204, 2007

8. Paik S, Shak S, Tang G, et al: A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 351:2817-3826, 2004

9. Goss PE, Ingle JN, Martino S, et al: A randomized trial of letrozole in postmenopausal women after five years of tamoxifen therapy for early-stage breast cancer. N Engl J Med 349:1793-1802, 2003

10. Kennecke HF, Olivotto IA, Speers C, et al: Late risk of relapse and mortality among postmenopausal women with estrogen responsive early breast cancer after 5 years of tamoxifen. Ann Oncol 18:45-51, 2007

11. Pestalozzi BC, Zahrieh D, Mallon E, et al: Distinct clinical and prognostic features of infiltrating lobular carcinoma of the breast: Combined results of 15 International Breast Cancer Study Group clinical trials. J Clin Oncol 26:3006-3014, 2008

12. Dubsky PC, Jakesz R, Mlineritsch B, et al: Tamoxifen and anastrozole as a sequencing strategy: A randomized controlled trial in postmenopausal patients with endocrine-responsive early breast cancer from the Austrian Breast and Colorectal Cancer Study Group. J Clin Oncol 30:722-728, 2012

13. BIG 1-98 Collaborative Group, Mouridsen H, Giobbie-Hurder A, et al: Letrozole therapy alone or in sequence with tamoxifen in women with breast cancer. N Engl J Med 361:766-776, 2009

14. Jin H, Tu D, Zhao N, et al: Longer-term outcomes of letrozole versus placebo after 5 years of tamoxifen in the NCIC CTG MA.17 trial: Analyses adjusting for treatment crossover. J Clin Oncol 30:718-721, 2012

15. Colleoni M, Giobbie-Hurder A, Regan MM, et al: Analyses adjusting for selective crossover show improved overall survival with adjuvant letrozole compared with tamoxifen in the BIG 1-98 study. J Clin Oncol 29:1117-1124, 2011

16. Goss PE, Ingle JN, Pater JL, et al: Late extended adjuvant treatment with letrozole improves outcome in women with early-stage breast cancer who complete 5 years of tamoxifen. J Clin Oncol 26:1948-1955, 2008

17. Burstein HJ, Prestrud AA, Seidenfeld J, et al: American Society of Clinical Oncology clinical practice guideline: Update on adjuvant endocrine therapy for women with hormone receptor-positive breast cancer. J Clin Oncol 28:3784-3796, 2010

18. van de Velde CJ, Rea D, Seynaeve C, et al: Adjuvant tamoxifen and exemestane in early breast cancer (TEAM): a randomised phase 3 trial. Lancet 377:321-331, 2011

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Lost in Translation? Estrogen Receptor Status and Endocrine Responsiveness in Breast Cancer

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See accompanying article on page 729

The question of when breast cancer is really estrogen receptor (ER) positive and endocrine responsive has troubled clinicians since the discovery of ER, but the issue is made even more acute by the fact that we can therapeutically alter the fate of some ER-positive breast cancers. Application of such therapies could be one size fits all, except that whereas endocrine therapy is generally considered to be well

tolerated, it can have an impact on quality of life, and specific adverse effects can even be life threatening. Thus, the optimal selection of patients for endocrine therapy depends on the appropriate integration of several factors. First, an accurate determination of receptor status is necessary; second, an assessment of the overall risk-to-benefit ratio for treatment in individual patients; and third, possibly an understanding of the degree of positivity.

This leads us to ask where the real threshold for ER positivity lies, and what is the optimal method of ER assessment? This, in turn, raises simple but important questions about the reliability and consistency of ER testing. Regan et al[1] demonstrated in tumor samples from International Breast Cancer Study Group (IBCSG) trials VIII and IX (571 premenopausal patients; 976 postmenopausal patients) that concordance of hormone receptor status by immunohistochemistry and by enzyme-linked immunosorbent assay ranged between 74% (progesterone receptor [PR]) and 88% (ER). Karn et al[2] demonstrated a concordance of 93% between ER expression (cutoff definition by bimodal distribution curve) and ER status in 3,030 U133A microarrays (Affymetrix, Santa Clara, CA) from breast cancer samples with known immunohistochemical or biochemical ER status. Harvey et al[3] showed, in tumors with low ER expression (1% to 10%), that immunohistochemical receptor assessment indicates a more favorable disease-free survival than the ligand-binding assay. Immunohistochemistry thus seems to be superior to biochemical determination, but the question remains whether this is merely attributable to methodologic issues or also to different underlying biologic properties. On the basis of these and similar data, most current guidelines set the threshold for endocrine responsiveness at ER expression of 1% or greater.[4,5] However, this does not address the variable treatment sensitivity of tumors that are above the threshold for positivity. In other words, a positive result is not an assurance of treatment sensitivity.

Whether and to what extent our definitions of positive or negative steroid hormone receptor status also reflect the biologic reality of endocrine responsiveness is questionable, because prospective data from clinical trials addressing this narrow but important issue do not exist. This is especially true for the small subgroup of low ER-expressing tumors, for which it seems that ER as a single marker loses its prognostic strength. Here, it may be particularly useful to add methods (eg, reverse transcriptase polymerase chain reaction) and complementary markers (eg, gene signatures or urokinase-type plasminogen activator/plasminogen activator inhibitor type 1) to enable a more accurate determination of the marker itself or to redefine endocrine responsiveness, given that the risk-to-benefit ratio for treatment in such patients is narrowed.

Even in higher risk cohorts (usually defined anatomically), breast cancer heterogeneity continues to pose a major problem, both in terms of our biologic understanding of the disease and in terms of our ability to predict outcome (prognosis) as well as therapeutic benefits (prediction). Conventional parameters such as ER, PR, human epidermal growth factor receptor 2, nodal status, tumor size, and grade provide important additional information. Yet global molecular techniques such as gene expression analysis have fundamentally changed our understanding of the disease.[6] We even expect that newer techniques will be added in the near future, such as next-generation sequencing. By identifying an intrinsic gene signature, ER-positive breast cancers can be classified into different prognostic subtypes. Sotiriou et al[7] showed that the search for subgroups provides important additional insights: Breast cancers with intermediate histopathologic grade can be divided with about half of the tumors expressing molecular markers associated with well-differentiated carcinomas and the remaining tumors expressing markers of poor differentiation. This subdivision is essentially accomplished by proliferation markers. Loi et al[8] showed that using a genomic grade index, ER-positive molecular subgroups can be defined that are closely related to luminal A and B tumors. Furthermore, Creighton et al[9] showed that the subgroup of ER-positive, PR-negative breast cancer can also be subdivided by gene expression analysis: Some tumors displayed a gene expression profile associated with either double-positive or double-negative tumors, and many had similarities with luminal B tumors. By applying proliferation-associated markers, ER-positive breast cancers can be subdivided into clinically highly relevant prognostic subgroups, although the predictive value (in terms of treatment response) remains unclear.

In the article that accompanies this editorial, Iwamoto et al[10] attempt to further define the endocrine responsiveness of low ER-expressing (1% to 9%) early breast cancers. In the largest series published to date (n = 465), they demonstrate the use of gene expression analysis; in those low ER-expressing breast cancers (n = 25), at least a quarter showed ESR1 mRNA expression, almost half were basal-like, and approximately 10% had luminal B characteristics by molecular definition (using the Prediction Analysis of Microarray [PAM50] classifier). None of these tumors showed now-classical features of luminal A tumors. Overall survival of the 1% to 9% ER-positive tumors ranged between those with $\geq$ 10% ER and the ER-negative tumors. Moreover, the authors applied the genomic index of sensitivity to endocrine therapy[11] (SET; 165 marker genes associated with ER expression) and showed that all tumors with ER 1% to 9% staining had low predicted endocrine sensitivity. Given the fact that SET was identified from genes coexpressed with ER, it seems likely that low ER and low ER expression also predict with high probability nonresponse to endocrine therapy using SET. SET was identified in patient cohorts that had received tamoxifen alone or subsequent to chemotherapy. Unfortunately, the clinical data from this retrospective single-center study does not allow more advanced treatment interaction analyses with respect to endocrine responsiveness, given that only 16% (n = 4) of the patients with 1% to 9% ER received adjuvant endocrine therapy. Using PAM50, 32% of the tumors were assigned to the human epidermal growth factor receptor 2 subgroup and 8% to the luminal B subgroup, and 12% were normal-like. Unfortunately, not enough clinical information is available regarding prediction of the treatment effect for these subtypes. For some molecular subtypes, the predictive value of an intrinsic gene signature has been investigated for response to neoadjuvant chemotherapy,[12,13] but data regarding differential responsiveness of intrinsic subtypes to endocrine therapy are still lacking. To date, the most important predictive discriminator in ER-positive breast cancer seems to be proliferative activity, which Iwamoto et al[10] only indirectly assessed by intrinsic subtype analysis using PAM50. Clinically, it would be interesting to know whether molecular testing by recurrence score,[14] which is only validated for ER-positive breast cancer, provides additional information in tumors with 1% to 9% ER expression.

Unfortunately, prospective data regarding treatment efficacy in breast cancers with low (1% to 9%) ER expression are not available. IBCSG demonstrated by Subpopulation Treatment Effect Pattern Plot analysis in Trial IX that postmenopausal women with node-negative

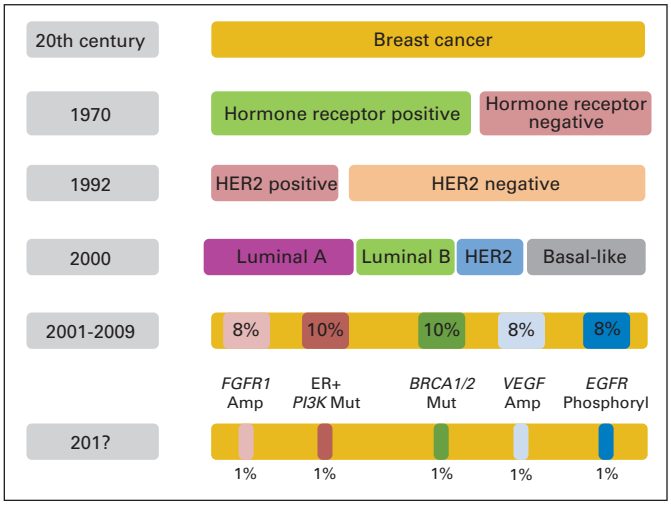

Fig 1. Timelines of biologic breast cancer subclassification: schematic overview. Amp, amplified; ER, estrogen receptor; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; mut, mutated; phosphoryl, phosphorylated; VEGF, vascular endothelial growth factor.

disease and ER concentrations of less than 10 fmol/mg cytosol protein substantially benefitted from adjuvant chemotherapy in addition to tamoxifen.[15] Khoshnoud et al[16] recently reanalyzed the Stockholm Adjuvant Tamoxifen Trial comparing immunohistochemical with biochemical ER determination in a low-risk group; the 1% to 9% ER-positive subgroup (n = 7) was too small for statistical analysis. In a recent meta-analysis, the Early Breast Cancer Trialists' Collaborative Group demonstrated that tamoxifen was ineffective in tumors with low ER expression (< 10 fmol/mg). Above this cutoff, however, significant therapy efficacy was seen, but additional determination of PR expression yielded no additional information for prediction of benefit from endocrine therapy.[17] In a recent issue of *Journal of Clinical Oncology*, Welsh et al[18] identified a subgroup of immunohistochemically ER-negative tumors that were ER positive by quantitative immunofluorescence and were associated with an outcome comparable with immunohistochemically ER-positive tumors. In the corresponding editorial, Wolff and Dowsett[19] stated that immunohistochemistry as the standard method of determining ER expression is not really standardized and that comparison of different methods is not informative in the establishment of a unique threshold value. The recent American Society of Clinical Oncology/College of American Pathologists testing algorithm, however, provides an important methodologic basis for clinical decision making.[20]

Overall, the empirical data suggest that there is no clear, methodologically consistent threshold for ER expression but rather a biologic threshold for ER expression associated with endocrine responsiveness. Furthermore, this threshold for responsiveness may be better determined by assessing molecular characteristics and properties than by mere quantification of ER expression. Beyond the known variation in routine methodology for ER assessment, this could be a sufficient explanation for the clinical observation of those few patients with low levels of ER expression who benefit from endocrine therapy, such as those with immunohistochemically ER-positive levels of 1% to 9%. Biology matters, and it is more than simply expression of ER.

In summary, Iwamoto et al[10] have taken us another important step beyond a purely descriptive breast cancer definition and toward the use of more biologically defined subtyping. Because of the small sample size and the lack of informative clinical data, their results can currently be considered as hypothesis generating. Despite their molecular findings, the authors still cautiously and appropriately recommend endocrine therapy for this subgroup. In principle, their retrospective results would now need to be validated prospectively, particularly with regard to clinical endocrine responsiveness. Yet, given the small percentage of low ER-positive breast cancers and the practice-changing molecular subtype paradigm, we may not be able to afford to move forward with such small steps. We should instead use this evidence to design trials today that will help to answer tomorrow's questions. Considering the accelerating rate of progress in biologic breast cancer subclassification (Fig 1), we should learn as much from past experience as possible to apply today's molecular findings to forward-looking trials and subsequently to modern therapy concepts. Refined requirements for obtaining high-level evidence from retrospective data analysis have already been put forward for biomarker development.[21] The thorough correlation analyses by Iwamoto et al, together with other retrospective data sets, may thus remain the best foundation regarding ER-low breast cancers that we will be able to efficiently generate.

Today, breast cancer heterogeneity needs to be elucidated using modern molecular biologic techniques that are already available[22] and

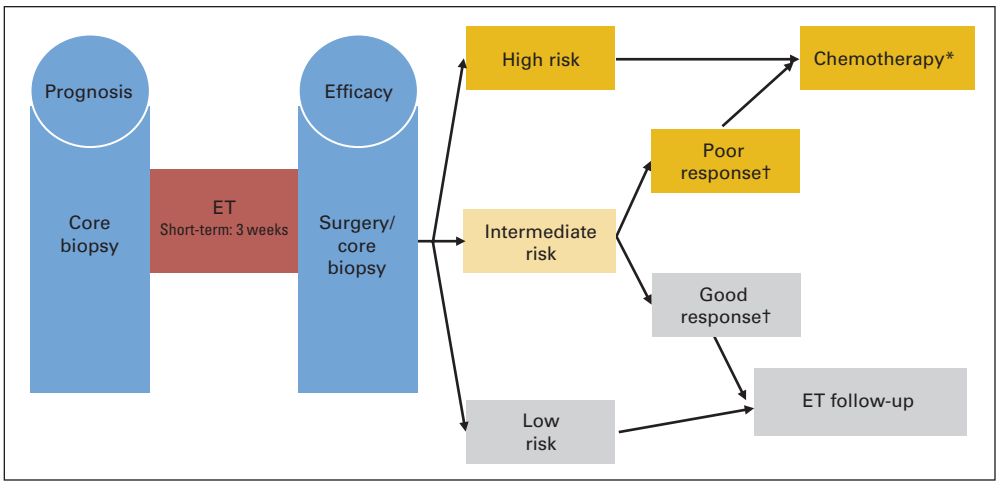

Fig 2. Women's Healthcare Study Group AM06 Adjuvant Dynamic Marker-Adjusted Personalized Therapy (ADAPT) trial (European Union Drug Regulating Authorities Clinical Trials No. 2011-001462-17). Subprotocol in hormone-receptor positive disease. (*) Followed by sequential standard adjuvant endocrine therapy (ET). (†) To short-term ET.

then addressed by innovative clinical trial concepts. In ER-positive breast cancer, we need to get past the era of large trials that compare treatment A versus B in unselected cohorts and instead perform practice-changing trials that tailor therapy to the biology of luminal A and B tumors, for instance.[23] Perhaps even in vivo assessment of endocrine responsiveness will be required to augment baseline assessment, such as was already implemented in the American College of Surgeons Oncology Group Phase II/III Randomized Study of Neoadjuvant Therapy Comprising Exemestane Versus Letrozole Versus Anastrozole in Postmenopausal Women With Estrogen Receptor Positive Stage II or III Breast Cancer (ACOSOG Z1031 trial), the POETIC study, and the Adjuvant Dynamic Marker-Adjusted Personalized Therapy (ADAPT) trial optimizing risk assessment and therapy response prediction in early breast cancer (Fig 2).

Together with our patients, we need vision and courage to embark on a journey toward such yet unknown destinations, always carefully weighing evidence as well as risks and benefits. The present study provides even more confirmation that this is important.

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REFERENCES

1. Regan MM, Viale G, Mastropasqua MG, et al: Re-evaluating adjuvant breast cancer trials: Assessing hormone receptor status by immunohistochemical versus extraction assays. J Natl Cancer Inst 98:1571-1581, 2006

2. Karn T, Metzler D, Ruckhäberle E, et al: Data-driven derivation of cutoffs from a pool of 3,030 Affymetrix arrays to stratify distinct clinical types of breast cancer. Breast Cancer Res Treat 120:567-579, 2010

3. Harvey JM, Clark GM, Osborne CK, et al: 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 17:1474-1481, 1999

4. National Comprehensive Cancer Network: Breast. http://www.nccn.org

5. Arbeitsgemeinschaft Gynäkologische Onkologie: Recommendations of Gynäkologische Onkologie Commission Mamma. http://www.ago-online.org/index.php?site=mamma_guide_topical&lang=de

6. Perou CM, Sørlie T, Eisen MB, et al: Molecular portraits of human breast tumours. Nature 406:747-752, 2000

7. Sotiriou C, Wirapati P, Loi S, et al: Gene expression profiling in breast cancer: Understanding the molecular basis of histologic grade to improve prognosis. J Natl Cancer Inst 98:262-272, 2006

8. Loi S, Haibe-Kains B, Desmedt C, et al: Definition of clinically distinct molecular subtypes in estrogen receptor-positive breast carcinomas through genomic grade. J Clin Oncol 25:1239-1246, 2007

9. Creighton CJ, Kent Osborne C, van de Vijver MJ, et al: Molecular profiles of progesterone receptor loss in human breast tumors. Breast Cancer Res Treat 114:287-99, 2009

10. Iwamoto T, Booser D, Valero V, et al: Estrogen receptor (ER) mRNA and ER-related gene expression in breast cancers that are 1% to 10% ER-positive by immunohistochemistry. J Clin Oncol 30:729-734, 2012

11. Symmans WF, Hatzis C, Sotiriou C, et al: Genomic index of sensitivity to endocrine therapy for breast cancer. J Clin Oncol 28:4111-4119, 2010

12. Rouzier R, Perou CM, Symmans WF, et al: Breast cancer molecular subtypes respond differently to preoperative chemotherapy. Clin Cancer Res 11:5678-5685, 2005

13. Rody A, Karn T, Solbach C, et al: The erbB2+ cluster of the intrinsic gene set predicts tumor response of breast cancer patients receiving neoadjuvant chemotherapy with docetaxel, doxorubicin and cyclophosphamide within the GEPARTRIO trial. Breast 16:235-240, 2007

14. Paik S, Shak S, Tang G, et al: A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 351:2817-2826, 2004

15. International Breast Cancer Study Group (IBCSG): Endocrine responsiveness and tailoring adjuvant therapy for postmenopausal lymph node-negative breast cancer: A randomized trial. J Natl Cancer Inst 94:1054-1065, 2002

16. Khoshnoud MR, Löfdahl B, Fohlin H, et al: Immunohistochemistry compared to cytosol assays for determination of estrogen receptor and prediction of the long-term effect of adjuvant tamoxifen. Breast Cancer Res Treat 126:421-430, 2011

17. Early Breast Cancer Trialists' Collaborative Group (EBCTCG), Davies C, Godwin J, et al: Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: Patient-level meta-analysis of randomised trials. Lancet 378:771-784, 2011

18. Welsh AW, Moeder CB, Kumar S, et al: Standardization of estrogen receptor measurement in breast cancer suggests false-negative results are a function of threshold intensity rather than percentage of positive cells. J Clin Oncol 29:2978-2984, 2011

19. Wolff AC, Dowsett M: Estrogen receptor: A never ending story? J Clin Oncol 29:2955-2958, 2011

20. Hammond ME, Hayes DF, Dowsett M, et al: American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol 28:2784-2795, 2010

21. Simon RM, Paik S, Hayes DF: Use of archived specimens in evaluation of prognostic and predictive biomarkers. J Natl Cancer Inst 101:1446-1452, 2009

22. Kaufmann M, Pusztai L, Biedenkopf Expert Panel Members: Use of standard markers and incorporation of molecular markers into breast cancer therapy: Consensus recommendations from an International Expert Panel. Cancer 117:1575-1582, 2011

23. Harbeck N: Struggling with subtypes: Trying to bridge the gap between molecular breast cancer subtypes and clinical management. Breast Cancer Res Treat 130:421-423, 2011

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